A COMPARATIVE STUDY OF LECITHIN/SPHINGOMYELIN RATIO & BUBBLE STABILITY TEST IN AMNIOTIC FLUID TO ASSESS FETAL LUNG MATURITY IN NORMAL & ABNORMAL PREGNANCIES

THESIS FOR

M. S. (OBSTETRICS AND GYNAECOLOGY)

BUNDELKHAND UNIVERSITY, JHANSI

1984 KAMLESH MAHESHWARI

CERTIFICATE

This is to certify that the work entitled "A COMPARATIVE STUDY OF LECITHIN/SPHINGOMYELIN RATIO AND BUBBLE STABILITY TEST IN AMNIOTIC FLUID TO ASSESS FETAL LUNG MATURITY IN NORMAL AND ABNORMAL PREGNANCIES", which is being submitted as a thesis for M.S. (Obstetrics and Gynaecology) by Dr. Kamlesh Maheshwari, has been carried out under my supervision and guidance in the department of Obstetrics and Gynaecology.

She has put in the necessary stay in the department as per university regulations.

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APRIL 30th, 1983. (KAMLESH MEHRCHANDANI)

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INTRODUCTION

INTRODUCTION

Determination of fetal maturity before delivery, forms a very important factor in modern obstetrics. The quality of perinatal life is well known to be dependent on genetic input, maternal environment, gestational age and better weight and it is further modified by intrapartal and neonatal events. More recently as one of the first major advances made in perinatal medicine, there has been the further recognition that fetal biological maturity, apart from gestational age and weight, is also essential to a safe transition through the crisis of birth and the new born maturity. Perinatologists have gained control on timing of both and become critical to have a reliable prenatal prognostic index of fetal pulmonary maturity.

The perinatal mortality is directly influenced by fetal lung maturity at the time of delivery. Prematurity, postmaturity and intrauterine growth retardation contribute highly to neonatal mortality and morbidity as well as subsequent mental retardation. Thus just more than 1/3 of perinatal deaths including almost half of the first week neonatal deaths attribute to immaturity and most of this mortality was due to

pulmonary immaturity leading to respiratory distress syndrome and hyaline membrane disease (Claireaux, 1953).

Hence prediction of gestational age and fetal lung maturity is essential when delivery is contempted before term in high risk pregnancies to prevent both delivery of premature infant as well as respiratory distress syndrome. This assumes significant clinical importance especially when details of last menstrual period is unreliable because of conception occuring during lactational amenorrhoea coupled with patients coming late for antenatal check-up or because of irregularity in menstruation.

The use of amniocentesis for diagnostic purpose is relatively new. The first reference to its diagnostic use was that of Nemeso et al (1930) who reported placental localisation by amniography. Since then there has been an exponential increase in the use of this technique and amniocentesis is now a standard approach in the evaluation of the fetus.

An accurate means of evaluating fetal maturity has always been tough. In recent years, a number of tests have been reported in medical literature for general fetal maturity and pulmonary maturity. Some of these are cytological examination of amniotic fluid, volume estimation, amniotic fluid creatinine level, spectrophotometric analysis of amniotic fluid, bilirubin

level of amniotic fluid, urea level of amniotic fluid and phospholipids level etc.

The most important current need in assessing the fetus is a simple procedure to provide reliable information about the degree of pulmonary maturity. The lecithin concentration and L/S ratio in amniotic fluid may be useful for fetal pulmonary maturity (Clunk et al, 1971, Nelson, 1972, Donald et al, 1973 and Bhagwanani et al, 1973).

In 1972 Clements et al reported a rapid, simple inexpensive bed side test using the minimum of apparatus and materials with high predictive value for the respiratory distress syndrome i.e. Bubble stability test (the foam or shake test or rapid surfactant test).

Fetal maturity embraces three essentially indepent processes -

1. Simple chronological process of increasing gestational age.
2. The growth of fetus in terms of increasing gestational age.
3. Functional maturation - signifying physiological development of fetal tissues and systems.

This physiological maturation determines viability of the fetus. And it is upon the functional capacity of

the lungs, rather than other organs, that the live born baby's survival depends.

Prior to 34 weeks of gestation, the principal indications for amniocentesis are detection of fetal hemolytic disease, genetic abnormalities and for congenital abnormalities. Between 34 to 40 weeks the main indication is for determination of pulmonary maturity. Beyond 40 weeks, amniocentesis is primitive to detect meconium staining as well as being indicator of fetal maturity disturbances (Wagstaff et al, 1973).

As surfactant (phospholipids) originate from the fetal lung their estimation in amniotic fluid would be indicator for fetal lung maturity. The surfactants because of its unique variable surface tension effect, when compressed prevent atelectasis and collapse of the alveoli at the end of expiration and thereby maintaining expansion of the alveoli on inspiration.

Gluck et al (1967) has found that this surface active substance is abundant in neutral lipids and phospholipids specially lecithin, perhaps lecithin rich material could be detected before term by amniocentesis. Prior to alveolar stability (about 35 weeks gestation) the ratio of lecithin to sphingomyelin is less than or equal to one. A ratio more than 2:1 indicates that a baby born at that point would not develop respiratory distress syndrome.

Babies born without this protective coating may develop respiratory distress syndrome (RDS). In this situation, alveolar surface will be elevated after expiration causing alveoli to collapse and inducing progressive atelectasis.

Though there are so many test to assess fetal maturity, still no single test is completely reliable and combination of 2 or more techniques are of greater value. Thus the present study is to evaluate the importance of bubble stability test and L/S ratio in amniotic fluid for the antenatal prediction of the fetal pulmonary maturity and the potential risk of RDS and assessing their correlative comparitive value.

****

REVIEW OF LITERATURE

REVIEW OF LITERATURE

In 1903, Hochheim first described hyaline membrane disease in the lungs of two infants who had died of respiratory difficulties. Subsequently to these findings this syndrome was called the hyaline membrane disease until 1959, when it was replaced by the term respiratory distress syndrome (RDS) (Vapaavuori, 1971).

Pulmonary surfactant :

1. History :

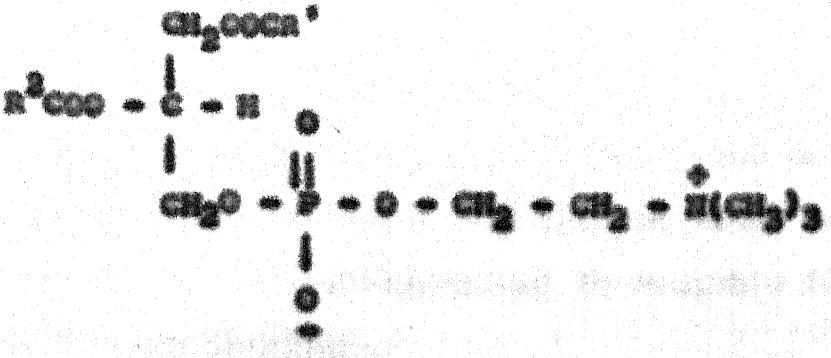
By studying the lung washing film Clements et al (1957) suggested that the stability of alveoli during expiration is due to surface tension lowering property of surfactants that prevents the complete collapse of alveoli. Clements et al (1957) identified the presence of surfactant in the lung tissue. The concept of Pattle (1958) of deficient surface active material in the alveolar lining as a cause of progressive atelectasis of hyaline membrane disease was confirmed by Avery and Mead (1959).

Kimua et al (1961) reported the isolation of a surface tension lowering substance, a surfactant, from bovine lung tissue and showed that this surfactant had

the ability to change surface tension. This surface active material was identified as complex lipoprotein (Pattle and Thomas, 1961), primarily composed of phospholipids like dipalmitoyl lecithin, later other surface active components were also purified and characterised from amniotic fluid (AF) and tracheal secretions of new born infants (Brown et al, 1975).

2. Chemistry of Surfactant :

The surfactant is a complex mixture of lipids, proteins and carbohydrates. Of these lipids, 90-95 per cent are phospholipids the rest being mainly cholesterol. The major surface active component is dipalmitoyl lecithin (DPL), which comprises about 50% of total surfactant lipids (Abrams, 1966 and Frosolono, 1970). It has a bipolar configuration, a hydrophilic choline group and two hydrophilic saturated fatty acid side chains. The addition of an organic base choline to phosphatidic acid yields the resulting molecule of lecithin :

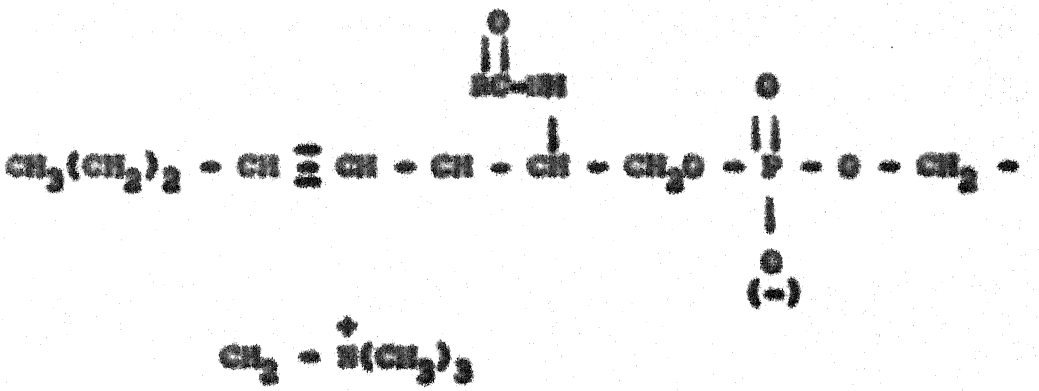


The second major component is phosphatidyl glycerol (PG) consisting of about 7-14 per cent of the

total phospholipids. In the adult type of surfactant, the minor component include phosphatidyl inositol, sphingomyelin, Phosphatidyl thanolamine, phosphatidyl serine and lysolecithin which accounts for 6-12% of phospholipids (Gluck et al 1967a, b and Hallman and Gluck, 1977).

Two surface active lecithin have been clearly identified, one is alphapalmitic betapalmitic acid lecithin or dipalmitoyl lecithin, the other is alphapalmitic betamyristic acid lecithin.

Sphingomyelin, a sphingo-phospholipid with sphingosine as alcohol (ceramide-1-phosphoryl choline)



Like lecithin each molecule of sphingomyelin contains one atom of phosphorus and one choline group. This molecule is important because Gluck (1972) chose the use of the sphingomyelin in amniotic fluid as a reference compound.

3. Biosynthesis :

Type II alveolar cells, also known as duct cells, niche cells or granular pneumocytes form the source of surfactant synthesis and secretion (Avery et al, 1973 and Benner, 1976). The principal surfactant components are probably synthesised in the endoplasmic reticulum and stored in lamillar inclusion bodies in the cytoplasm of these cells (Morgan, 1971 and Hallman and Gluck, 1977).

Lecithins are synthesised in lung through two major pathways. The choline incorporation pathway is the most important in the de novo synthesis of total lecithin, accounting for 90% of lung lecithin synthesis in late pregnancy (Epstein and Farrell, 1975, Hallman et al, 1977). This pathway is present in the lungs of human fetuses of 18-20 weeks of gestation, at which time only small amount of DPL are produced. The activity of this pathway increases slowly to 35th or 36th week of gestation after which a surge in its activity can be seen.

The methylation pathway always seems to remain less active than the choline incorporation pathway (Gluck et al, 1967 a, b). The methylation pathway is sensitive to hypoxia, hypothermia and hyperoxia (Gluck et al, 1972 and Smith and Tooley, 1974), but is unaffected by acidosis (Merritt and Farrell, 1976).

4. Surfactant in Amniotic Fluid :

The fetal lung functions as a secretory organ and participates in amniotic fluid (AF) formation (Adams et al, 1967). Enhorning and Adams (1963) reported that 0.02 - 0.013 ml/kg per minute of fluid is produced in respiratory tract of fetus. Fetal lungs contribute fluid and surface active lecithin to amniotic fluid in late pregnancy (Biggs et al, 1973). The surface active lecithin in the pulmonary secretions correspond closely to the amount of these lecithin present in the alveolar lining (Biggs et al, 1974).

A part of lecithin in amniotic fluid arises from other sources besides the fetal lung (Abramovich et al, 1975). These include fetal skin, urine, serum, fetal membrane and placenta (Condorelli et al, 1974).

The amount of surface active lecithin in amniotic fluid accurately reflects the concentration of surfactant in the alveoli (Gluck and Kulovich, 1972). In early pregnancy the concentration of lecithin in amniotic fluid is very small, consisting at the 20th week of gestation of 21.0 per cent of the total phospholipids, while the proportion of sphingomyelin at that time is 51.3 per cent (Hieaemshi, 1973). After 35th week of gestation, a sharp rise in lecithin concentration is seen, reflecting a surge in the activity of the choline incorporation pathway (Bryson et al, 1972 and Gluck &

Kulovich, 1973). The concentration of sphingomyelin is very stable and no significant changes are seen between 32 and 40 weeks gestation (Roux et al, 1973 a, b). Before term the proportion of lecithin and sphingomyelin in the total phospholipids are about 65% and 8% respectively (Arvidson et al, 1972 and Diamanshi, 1973).

According to Ousden and Waite (1972) there was approximately a two fold increase in the total phospholipid concentration between the first trimester and term. Whereas lecithin accounted for between 28 and 51% of the total phospholipid during first half of pregnancy, at term this proportion had increased to between 50 - 79%. The contribution of sphingomyelin however fell from 29 - 51% to 25 - 46%. The concentration of lecithin increased from 0.44 mg/100 ml at the early gestation to 2.90 mg/100 ml at term. The concentration of sphingomyelin on the other hand fell from 1.07 mg/100 ml to 0.35 mg/100 ml.

Phosphatidylinositol increases slowly parallel to L/S ratio after the 32nd week of gestation, until this reaches to 42 weeks gestation, when it disappears. Phosphatidylglycerol (PG) appears during 35 to 38 weeks of gestation and has a good linear correlation with the L/S ratio thereafter (Hallman and Gluck, 1977 and Hallman et al, 1977).

Lecithin comprises almost 80% of the surfactant phospholipid (Thelma et al, 1980).

5. Methods for Amniotic Fluid Surfactant Estimation :

In 1969, Nelson suggested that decreased proportion of lecithin in the total phospholipids of amniotic fluid might be associated with infants developing RDS. Since then several methods of amniotic fluid analysis have been found beneficial in predicting RDS.

A new method for the antenatal prediction of RDS was developed by Gluck et al (1971), which is now-a-days the most widely employed with numerous modifications, namely the L/S ratio. Other tests for AF surfactant include the measurement of lecithin phosphorus (Nelson, 1972, 1975), lecithin concentration (Bhagwanani et al, 1972 a, b), total AF lecithin (Caspi et al, 1975), total phospholipids (Gluck and Waite, 1972), estimation of AF palmitic acid (MacLennan et al, 1975), the palmitic acid/stearic acid ratio (Schirar et al, 1975) and the percentage of stearic acid (Frantz et al, 1973).

Recently a rapid, inexpensive and simple screening test has been developed by Clements et al (1972), i.e. the bubble stability test (the foam test or shake test).

Bubble Stability Test, Pulmonary Maturity and Risk of RDS :

Clements et al (1972) proposed the significance of foam stability test (FST) for assessment of fetal pulmonary maturity and observed that all the infants

with clearly negative FST had severe respiratory distress syndrome (RDS) or transitional respiratory distress (TRD), whereas all the infants with clearly positive test were free of respiratory distress. In 13 cases with intermediate test, 8 infants showed mild to severe respiratory difficulties and rest were free of respiratory difficulties.

Roux et al (1972) found that the shake test was always positive when the lecithin phosphate concentration was greater than 180 µg/100 ml. These authors determined that for values of lecithin phosphorus encountered at 36 weeks, the incidence of false negative shake test is 26.7%.

Nobel et al (1972) performed the shake test in 65 cases of pregnancies at various gestation. Test was positive in 42 cases and none of these cases developed RDS. The test gave intermediate results in 12 cases. Of these 4 developed RDS, 2 had non-defined respiratory distress and 5 did not have any respiratory difficulty. Eleven cases were having negative test and all of them developed respiratory distress.

Attempts have been made by Pozzard and White (1972) to predict the potential risk of RDS in newborn by measuring bubble stability. They found that bubble stability in amniotic fluid increased from early to late pregnancy.

Edwards and Baillie (1973), Dubowitz (1973) and Roux et al (1973) observed that negative test was associated with high risk of RDS and clearly positive test signals a low risk of respiratory distress.

Fisher and Sutherland (1973) performed shake test in 86 cases at 34 to 42 weeks of gestation. Fifty cases have positive shake test, 17 cases with intermediate and 11 cases with negative test result. Out of 86 cases, only one neonate (34 weeks gestation) with a negative test developed RDS. They observed interesting finding of false negative results in eleven cases of hydr-amnios. They have reported one still birth case with negative test associated with pulmonary hypoplasia and multiple congenital abnormalities.

Parkinson et al (1973) observed bubble stability clicking in samples of amniotic fluid. The supernatant fluid after centrifugation was first examined with the "shake test" (Clements et al, 1972). Samples of these bubbles in deaerated water, were examined under microscope and all demonstrated the bubble clicking phenomenon. This phenomenon appears to be characteristic of bubble lined with surface active material obtained from the lungs. This test may form the basis of a useful test for pulmonary maturity in human fetus.

Roux et al (1973 b) observed the result of foam test in 44 cases of high risk pregnancy (34 to 40 weeks).

In 44 delivered cases, 31 of positive results were mature and at term, 9 had intermediate result and also had no RDS. In 5 cases of negative FST result, 2 (40%) had hyaline membrane disease (HMD) (28 and 34 weeks) and one (20%) had respiratory distress (36-37 weeks). The data suggested that positive test is cent per cent accurate in predicting a mature fetus, an intermediate can be observed in premature infants without lung complications as well as in near term fetus. A negative test indicates a premature fetus and risk of HMD may be high.

Bhagwanani et al (1973) observed the result of the bubble stability test (BST) in 80 cases (35 to 42 weeks of gestation). The test was positive in 37 cases indicating cent per cent normal fetal maturity as none of these 37 cases developed RDS. The test was intermediate in 18 cases and negative in 25 cases. Six cases (24%) in negative group and 2 cases (11%) in intermediate had RDS.

Goldstein et al (1974) observed 12% of the infants with a transitional or immature shake test developed RDS. They further suggested that values of shake test may be as a screening test for the fetal pulmonary maturity especially when a mature pattern is found.

Thibeault and Nobel (1974) performed bubble stability test (BST) in 102 cases of high risk pregnancies

and observed that RDS was in 36% (20 of 56 cases) of pregnancies with positive BST results, with intermediate BST 54% (14 of 26 cases), with negative 96% (23 of 24 cases). The incidence of RDS, however was significantly related to negative BST. The case fatality rate (CFR) was not significantly related to bubble stability rate. The CFR was significantly related to low 5 minutes Apgar score, suggesting that the events prior to delivery contribute towards this increased mortality rate. RDS also occurred in immature infants with positive BST and good Apgar score which indicates that immaturity is independent variable in the pathogenesis of RDS. There are 3 prime important factors in the pathogenesis of RDS e.g. surfactant deficiency, intrapartum complications and immaturity. RDS may occur more frequently in infants of below 34 weeks gestation. This indicates that immaturity is one of the cause of RDS.

Rothbard (1974) performed foam test (FT) in 82 consecutive high risk pregnancies and observed that positive FT cases had no RDS, though negative FT is not always conclusive (i.e. may be false negative). Patients with intermediate value may develop transitional respirative distress but usually do not progress to hyaline membrane disease.

Sproule et al (1974) performed bubble stability test (BST) in 356 samples of amniotic fluid. They

observed that BST was useful in predicting the risk of neonatal respiratory distress. A positive test at 1 : 2 dilution indicates that respiratory distress is most unlikely to occur if baby is delivered without delay, but a negative result calls for a more direct measurement of fetal pulmonary surfactant in amniotic fluid e.g. L/S ratio or lecithin concentration.

Cowett and Oh (1976) observed the result of bubble stability test (BST) in 109 cases of various gestation (35 - 42 weeks). Seventy nine of 81 (97.5%) infants with a positive BST had no evidence of respiratory distress (R.D.). The remaining 2 cases with positive test had transient respiratory distress (TRD). 20 infants who had intermediate test, 17 (85%) had normal respiration and 2 (10%) cases had TRD and one (5%) developed RDS. Out of eight infants with negative BST, 2 (25%) had no evidence of R.D., 4 (50%) had TRD and 2 (25%) had RDS.

There was slightly decreased production of surfactant in the high risk pregnancies for the identical gestational age group (35 - 38 weeks or above). Out of 99 cases (72 of normal pregnancy and 27 of high risk pregnancy), 12 had developed RDS, 7 of them belonged to normal group and 5 to high risk pregnancy. Two infants who had severe RDS, one had negative shake test and was of 36 weeks of gestation with hydramnios (Reddy et al, 1978).

Bhushan and Nirchandani (1978) have correlated the bubble stability with birth weight and maturity score. Negative BST is most likely in primitive fetus, it suggests gestation of 34 or less, predicts a new born with weight less than 2 kg (87.5% cases) and low maturity score (Dubowitz, 1970). They also observed that positive prenatal BST predicts birth weight more than 2.5 kg (87.5%) and a newborn with maturity score corresponding to one above 38 weeks pregnancy. In intermediate results, there was a wide scatter of both birth weight and maturity score.

Sharma et al (1981) found that shake test was more informative after 32 weeks gestation and can be used as screening procedure.

L/S ratio, Pulmonary Maturity and Risk of R.D.S. :

Estimation of ratio between lecithin and sphingomyelin is a widely used, quick, reliable, simple and careful rather than highly skilled laboratory technique for pulmonary surfactant and serves as a guide to fetal lung maturation.

After the detection of amniotic fluid lipids by Nieszenski et al (1960) and correlation of pre-maturity and respiratory distress with phospholipid constituent in amniotic fluid by Nelson (1969), it was Gluck et al (1971) who by reflection densitometry concluded that

terminal rise in amniotic fluid lecithin towards term is not matched by corresponding increase in sphingomyelin concentration.

A simple yet surprisingly accurate and reproducible method for L/S ratio determination, introduced by Gorer et al (1971), is based upon measurement (length x width) of chromatographed spots of lecithin and sphingomyelin area ratio (LSAR).

The predictive accuracy of the lecithin and sphingomyelin area ratio (LSAR) was soon confirmed by Whitfield et al (1972), who studied 2000 samples of amniotic fluid by planimetric method. As reported by Whitfield and Sproule (1974) in 466 cases LSAR was more than 2.0 but RDS occurred only in 3 of the babies, two of which were born to diabetic mothers and other was anaemic due to Rhesus incompatibility. Four fifth of the babies associated with dangerously low predelivery ratio (< 1.5) developed usually severe RDS, out of which about half died and one fifth of those with intermediate ratio (1.5 - 2.0) developed respiratory distress which was not usually severe and only one of 13 affected babies in this group died. These findings supported the contention that critical LSAR value using planimetric method is 2.0 rather than 1.8 as suggested by the originator (Gorer et al, 1971).

Lemons and Jaffe (1973) using Charring and visual interpretation technique for L/S ratio measurement in their series found the higher incidence of RDS despite

the ratio being greater than 2.0.

Gluck and Kulovich (1973) achieved 100% accuracy in predicting 30 instances in RDS from 51 amniotic fluid samples obtained not more than 24 hours before delivery. Using a critical ratio of 2.0 even with longer samples delivery interval (24 - 72 hours) there was no difficulty in relation to 9 out of 40 lower ratios, when the ratio was at least 2.0.

Cedard et al (1973) estimated L/S ratio in 185 amniotic fluid samples obtained during week preceding delivery including 170 samples obtained 3 days before delivery. No RDS detected in relation to 2.0 ratio, with intermediate value incidence was 12% but its incidence with low ratio (0.5 or less) was as high as 64%.

Donald et al (1973) reported 3.7% false positive results which were associated with maternal diabetes and or birth asphyxia. Nagetaff and Bromham (1973) found only one false positive result in their series of 100 cases.

Morrison et al (1974) found that L/S ratio of more than 2.0 indicates safely mature lungs in their series of 100 cases, using either visual assessment or densitometry.

Goldstein et al (1974) reported single false positive result in series of 400 predelivery tests using critical LSAA value as 2.0.

Kenisten et al (1975) reported L/S ratio measurement in 193 cases. Samples were obtained 72 hours before delivery. No RDS was detected with ratio value of 2.0 but with intermediate value (1.5) the incidence was 13%.

Olson et al (1975) determined the L/S ratio from measurements of the concentrations of these two phospholipids in 135 amniotic fluid samples. They found that predelivery ratio seems to be 3.5 since RDS did not occur in any of the 82 cases with higher values, but there was an increasing incidence of RDS in association with lower ratio value. RDS occurred in 6 out of 35 babies (17%) associated with ratios between 2.6 and 3.5 and in 9 out of 13 babies (69%) with ratios between 1.6 and 2.5. All 5 babies associated with ratios less than 1.5 died from RDS (100%).

Duhring and Thompson (1975) found the higher incidence of RDS despite the ratio being higher than 2.0 and associated with series of Lemons & Jaffe (1973) in both series together there were 12 incidence of RDS (2 fatal), among 205 cases with L/S ratio of more than 2.0. Seven of the affected babies were born to diabetic mothers, 3 had severe Rh-incompatibility, with lower ratios 11 out of 17 babies in these 2 series developed RDS.

Rome et al (1976) using LSAR technique took 2.0 as critical value because only one baby developed RDS

in 320 babies with higher values (2.1) born within 72 hours of collecting sample.

Cruz et al (1976) reported an increased risk of RDS in neonates with low Apgar score inspite of mature L/S ratio.

Tiwari et al (1979) found in series of 55 cases, mean L/S ratio 0.57 at 28 - 30 weeks, 2.30 at 35 - 36 weeks, 3.02 at 39 - 40 weeks and 3.45 at more than 40 weeks gestation and thus concluded that ratio of 2.0 and more than 2.0 indicated mature fetal lung.

O'brien and Cefalo (1980) found the predictive values of mature L/S ratio (2.0 or more) about 90% in normal pregnancy. But non-mature L/S ratio (less than 2) may predict RDS only in about 50% of cases. The accuracy rate of the L/S ratio was always highest, about 95 to 96% and a somewhat higher accuracy of 98.78% in the late trimester delivery group (Chieh-Lung-Chow, 1981 and Te-Lin-Lia, 1981). Similarly 94 - 98% accuracy has been reported by Cunningham (1981).

In a series of 246 cases Sharma et al (1981) related L/S ratio with different gestational periods. It was 0.096 at 26 to 28 weeks, at 29-31 weeks 0.818, 1.113 between 32 to 34 weeks, 2.207 between 35 to 37 weeks, 2.567 between 38 to 40 weeks and 3.016 between 41 to 43 weeks gestation.

Bubble Stability Test Vs L/S Ratio :

Clements et al (1972) showed a correlation between bubble stability (BST) and L/S ratio.

Parkinson and Harvey (1973) have compared L/S ratio and shake test in amniotic fluid and found statistically similar results of L/S ratio and shake test.

Frank H. Bouhm et al (1973) found in a comparative study of results of shake test and L/S ratio, an overall correlation rate of 64%. Clearly mature or immature rapid surfactant test gave the most predictable results. A mature shake test result was associated with an 80% accuracy when comparing this test to the L/S ratio.

Wellcome and Bronham (1973) compared the result of L/S ratio and bubble stability test for the estimation of surfactant in 190 sample of amniotic fluid of various gestation (12 to 42 weeks). the L/S ratio was high in 130 cases and was correlated by a positive test in 97 (74.6%) cases. In 32 cases, the high ratio was associated with an intermediate result and in one, the shake test was negative. L/S ratio was below 1.5 in 33 samples. In 31 cases, the shake test was negative and in 2 cases intermediate. A ratio below 2 was never associated with a positive shake test.

Goldstein et al (1974) have compared the value of L/S ratio and shake test for estimating fetal pulmonary

maturity. They found that shake test demonstrated less maturity than L/S ratio in 34 per cent and more maturity in 6.6 per cent of samples.

Lindback et al (1974) observed the relationship of bubble stability test (BST) and lecithin concentration in 60 cases. BST was dependent on concentration of lecithin. Risk of respiratory distress was associated more with low level of lecithin and negative BST.

Mukherjee et al (1974) evaluated amniotic fluid shake test and L/S ratio in 98 cases in the third trimester of pregnancy to predict fetal lung maturity. The L/S ratio, although mostly corroborative with clinical picture of babies, sometimes shows lower or higher values than expected, depending on the clinical condition of the mother. In this study they found 'shake test' superior to L/S ratio in predicting lung maturity. All the infants in this study who developed RDS showed a negative shake test.

Narcia et al (1974) performed foam test in 92 cases of normal and complicated pregnancies and compared the results with L/S ratio. They observed a linear relationship between L/S ratio and foam test.

Shephard et al (1974) reported the false positive rate of 18% in their study of comparison of bubble stability test & L/S ratio. It has usually been found that the bubble stability test (BST) rarely gives a

in disagreement with a still 'immature' L/S ratio), but false negative results (suggesting insufficient surfactant despite a 'mature' L/S ratio) are not infrequent (Wagstaff and Bromham, 1973; Goldstein et al, 1974; Marois et al, 1974; Thibeault and Nobel, 1974).

Cowett et al (1975) performed bubble stability test (BST) in 79 samples of gastric aspiration of neonates. The L/S ratio was determined in 27 samples. They suggested that BST on gastric aspirate may be reliable index for fetal pulmonary maturity, whose amniotic fluid is not available.

Sharma et al (1981) compared the three parameters L/S ratio, shake test and total phospholipid phosphorus (TPP) for assessment of fetal lung maturity. Their study suggested that TPP values are more reliable prior to 32 weeks when lecithin is low. While after that L/S ratio and shake test are more informative. Shake test can be used as a screening procedure and more elaborate L/S ratio can be estimated when very small bubbles are obtained or bubble clumping occure or in intermediate result, to differentiate the possible immature fetus from the likely mature fetus and in those cases showing a deviation from normal.

Factors Influencing Phospholipid level in Amniotic Fluid :

In predicting respiratory performance at birth, it is important to consider the factors influencing the critical intermediate ratio of lecithin and sphingomyelin.

Hobbins et al (1972) and Gluck et al (1974) confirm that contamination with blood may elevate L/S ratio to a mature level. Wagstaff et al (1974) found that with increasing concentration of maternal blood in amniotic fluid, there was a rise in the measured L/S ratio ($\geq$ 2.0), with as little concentration as 3.6 ml of whole blood per 100 ml of amniotic fluid. The shake test result also changed from negative to positive.

Fisher and Sutherland (1973) observed that excessive volume of amniotic fluid may influence the interpretation of shake test result and in predicting RDS.

In a series of 400 premature and mature newborn Gluck (1972) postulated that early methylation pathway of lecithin synthesis is inhibited by acute asphyxia and acidosis. Mature infants seems to be protected by the terminally active synthesis of dipalmitoyl lecithin which appears to be unaffected by hypoxia or acidosis.

Contamination of amniotic fluid with meconium interfers with the interpretation of chromatograms (Bryson et al, 1972 and Hobbins et al, 1972). Wagstaff et al (1974) demonstrated a consistent rise in the L/S ratio with increasing contamination of meconium. The shake test was made positive even when the contamination was not visually obvious.

Wagstaff et al (1974) demonstrated that in untreated amniotic fluid at room temperature apparent losses occurred in both the lecithin and sphingomyelin fraction over a period of 48 hours. After only 4.5 hour lecithin values were 19-29% less than their initial value and at 48 hour had fallen by 26-37%. Losses upto 23% occurred in sphingomyelin fraction over a similar period. The losses in samples stored at the ambient temperature or at -20°C were much smaller.

With increasing increments of bilirubin equivalent to 49 to 58 ug/100 ml added to amniotic fluid with L/S ratio of 1.3 and a negative shake test, no changes were observed in the L/S ratio or shake test results (Wagstaff et al, 1974).

Rothbard et al (1974) found false negative results of foam test in 2 cases of polyhydr-amnios who had no RDS. These false results were due to dilution factor of amniotic fluid. Reddy et al (1978) also observed false negative results in 2 cases of hydr-amnios who had no RDS.

Gluck and Kulovich (1973) reported delayed maturation of the L/S ratio in a significant number of babies born to mother with diabetes. Shreyer et al (1974) found normal values and trends for the L/S ratio in 31 samples from 15 diabetic mothers, several series confirmed that ratio remains static or declines in about one-third

of women with overt or latent diabetes. Polishuk et al (1974) described 5 such examples of a falling L/S ratio, but did not fall below 2.0 and there was no associated RDS. Dyson et al (1975) found generally normal L/S ratio in 148 samples from 71 diabetic mothers but there was a falling trend in 14 out of 35 series test with an associated increase in perinatal asphyxia and mortality.

Kenieton et al (1975) described 3 examples of subnormal L/S ratio in diabetics (1.0 at 35 weeks, 0.97 at 37 weeks and 0.8 rising in 3 days to 1.53 at 38 weeks) several days before the birth of babies (weighing 3300 gm or more) who developed RDS. Similar cases were reported by Mukherjee et al (1974) and Merola et al (1974).

Whitfield and Sproule (1974) found normal lecithin sphingomyelin area ratio (LSAR) and observed terminal rise in 150 cases of Rh incompatibility where baby was not severely affected. There was static or falling trend in association with 27 of 60 severely affected fetus (cord blood haemoglobin $\angle$ 11.0 gm/100 ml). However, in 2 cases of severe Rh disease, they found LSAR fell precipitously from well above the normal range to almost subnormal values. It seems possible that severe anaemia may induce an initial (possible cortisol induced) stress response in the fetus that stimulates the alveolar type II cells, but if an acute hemolytic crisis then occurs, surfactant synthesis may fail.

Lemons and Jaffe (1973) and Duhring and Thompson (1975) found normal L/S ratios in series of Rh disease patients.

In correlation with birth asphyxia Kulbac and Newman (1974), Duhring and Thompson (1975) and Kenniston et al (1975) found high incidence of RDS in babies delivered by caesarean section. This supports the view that respiratory distress is more likely to follow abdominal than vaginal delivery due to impaired replenishment of surfactant resulting from the acute asphyxia.

Fairbrother et al (1975) found higher concentration of pulmonary surfactant in amniotic fluid in malnourished fetus. Malnourished fetus had higher "Fat-cell" count in amniotic fluid than well nourished contemporary.

Gluck et al (1974), Dyson et al (1975) found a significant pulmonary maturation acceleration in condition such as maternal vascular disease, pre-eclampsia and repeated placental abruption.

Chiswick (1976) and Berkowitze et al (1976) found considerably low incidence of RDS in deliveries associated with rupture of membrane as compared to control group. And an increase in L/S ratio was shown.

Craven et al (1976) reported fluctuating amniotic fluid lecithin levels with a significant overall downward trend during labour.

Whittle (1977) has recently demonstrated very variable effect of labour on the L/S ratio. By a study on 48 cases, he found that ratio increased in half, remained same in one third, but fell in remaining. When there was rise, the increase was inversely related to duration of labour. In the assessment of lung maturity in diabetes mellitus both L/S ratio and palmitic acid concentration have proved unreliable (Rohlenberg et al, 1977; Hood et al, 1977 and Mulinar and Hunbank et al, 1978).

Bates et al (1979) reported altered L/S ratio in growth retarded fetus and Thomas et al (1980) found significantly higher L/S ratio in a 450 cases series of intrauterine growth retardation.

Cunningham (1981) reported 94 - 98% accuracy of L/S ratio in diabetic series.

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MATERIAL AND METHODS

MATERIAL AND METHODS

The present study was carried-out in the Departments of Obstetrics and Gynaecology and Biochemistry at Maharani Laxmi Bai Medical College & Hospital, Jhansi, during the period of June 1982 to March 1983.

Selection of cases :

The material for the study was selected from the admitted cases of pregnancy. Cases of pregnancy of various weeks of gestation varying from 18 to 40 weeks and above 40 weeks were taken for the study. The duration of pregnancy was calculated from last menstrual period and by clinical examination. Cases where determination of gestational age could not be done, were excluded.

On the basis of clinical presentation, the following groups were made :

(A) **Cases of Normal Pregnancy :**

In this group, cases who were not having any pathological signs and symptoms either during their antenatal period or at the time of delivery and also those normal cases of pregnancy who came for medical termination of pregnancy were included.

(B) Cases of Complicated Pregnancy :

In this group, following types of cases of pregnancy with complications were included.

1. Post maturity/dysmaturity.
2. Fetal distress.
3. Toxaemia of pregnancy :
   - Essential hypertension
   - Pre-eclampsia
   - Eclampsia.
4. Uterine bleeding :

   (Inevitable abortion and antepartum haemorrhage including placenta praevia and accidental haemorrhage).
5. Hydr-amnios.
6. Multiple pregnancy.
7. Hydrocephalus.
8. Heart disease.
9. Diabetes.
10. Rh immunisation.

Clinical Presentation :

1. History : Detailed history of present pregnancy was taken and precise date of last menstrual period was investigated to know the period of gestation.

   Past obstetrical history and family history of twins, hydr-amnios or any congenital abnormalities were also taken.

2. General Examination : Thorough general examination was done with special attention to pulse, blood pressure, pallor, oedema, jaundice and cyanosis.

3. Systemic Examination : of cardiovascular and respiratory system was done.

4. Per Abdomen Examination : was done to determine the approximate duration of gestation, position and condition of fetus. Fetal heart sounds were heard to know any evidence of fetal distress. Any evidence of twins, breech, transverse lie or hydr-amnios was also looked for.

5. Per Vaginum Examination : done to exclude contracted pelvis or cephalopelvic disproportion and for the dilation of cervix, progress of labour and to detect meconium.

6. Investigations :

Routine : Blood : HB, TLC, DLC
Urine : Albumin, Sugar.
Blood grouping : AB / A / B / O
Rh Positive/Negative.

Special : Blood sugar : Fasting and postprandial.
VDRL :
Blood urea:

Method of obtaining Amniotic Fluid (AF) :

1. Per vaginal.
2. Trans-abdominal.
3. During caesarian section and hysterotomy.

1. Per vaginum :

This is done through the bulging membranes in cases who are in labour after 2 finger di-latation of cervix with the help of needle and syringe with all aseptic measures. Precautions were taken not to contaminate the samples with vaginal discharges, urine or blood.

2. Trans-abdominal :

Abdominal examination was carried out to know the presentation and position of fetus. Fetal heart sounds were counted for regularity. Abdomen was cleaned with rectified spirit.

A point was taken midway between the fetal upper and lower limbs and of nape of neck. With all aseptic measures a 20 gauge, 4 inches spinal needle with stilette (with or without using local anaesthetic solution) was directly introduced into the uterus. Stilette was removed and 10-15 ml of liquor amnii was aspirated by a clean glass syringe. Then the needle was briskly removed and point was sealed with tincture benzoin. If no fluid was obtained after 2 puncture or if the bloody tap obtained, procedure was abandoned.

3. <u>During Caesarean Section And Hysterotomy</u> :

During caesarean section and hysterotomy, 10-15 ml of amniotic fluid was collected with the help of autoclaved syringe and needle after incising the visceral peritoneum.

<u>Storage of Samples</u> :

Amniotic fluid samples were stored in clean test tubes and labelled. Amniotic fluid was subjected immediately or if not it was refrigerated at 5°C within an hour. The samples that could not be analysed, the same day were frozen at -20°C.

<u>Baby's Examination</u> :

<u>General Appearance</u> :

Weight : Cyanosis : Jaundice :

Apgar Scoring (Apgar 1953) :

Heart rate :	Absent/slow	Below 100/minute Above 100/min.
Respiration :	Absent/slow	Irregular/regular, rate, good crying.
Muscle tone :	Limp/some flexion of extrimities/ active motion.	
Response to catheter :	No response/Grimace/Cough or sneez.	
Colour :	Blue/Pale/body pink, extrimities blue/ completely pink.	
Any other :		

<u>Respiratory Examination</u> : Any other abnormality

Chest X-ray (if evidence of RDS present).

Respiratory Distress Syndrome (RDS) :

Diagnosis of RDS was made on careful clinical and radiological examination of infants based on Silverman and Anderson Retraction Score (1956).

1. Clinical Features :

- Difficulty at birth shown by delayed onset of regular respiration.
- Respiratory rate below 60/minute.
- Xiphoid retraction.
- Expiratory grunt.
- Cyanosis.

Two cases of these items present at the time of examination more than an hour apart are sufficient to make diagnosis of RDS.

2. Radiological Features :

Diffuse reticulo-granular pattern, dispersed evenly throughout the lung field with increasing severity, granular areas increased and become confluent so that lung has appearance of ground glass and borders of heart obscured. Air bronchogram sign present. Thorax appears to be well expanded and ribs interspaces are broad.

Degree of RDS : determined by Silverman and Anderson Retraction Score (1956). With this criteria, infant is observed for 5 features -

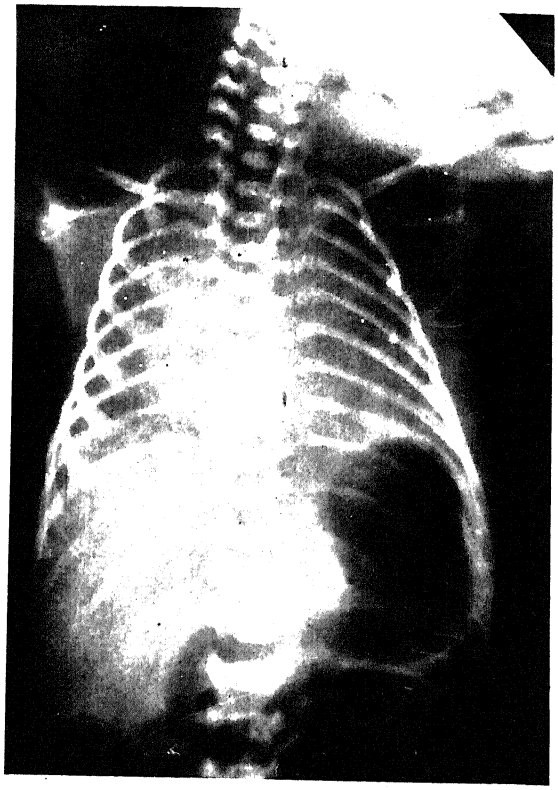

Fig. 1

PREPARATION

GASTRIC ASPIRATE or AMNIOTIC FLUID(ml)	1.00	0.75	0.50	0.25
SALINE (ml)	0.00	0.25	0.50	0.75
TUBE DILUTION	1/1	1/1.3	1/2	1/4
ALCOHOL 95% (ml)	1.00	1.00	1.00	1.00

1ml mixture + 1ml 95% alcohol = 47.5 % concentration

INTERPRETATION

POSITIVE TEST	3+ 3+	3+ 3+	3+ 2+	2+ 1+
INTERMEDIATE TEST	3+ 2+	2+ 1+	1+ 1+	0 0
NEGATIVE TEST	1+ 0	0 0	0 0	0 0

Fig. 2

Fig. 1 : X-ray chest of an infant who had R.D.S. showing diffuse reticulo-granular pattern and ground glass appearance.

Fig. 2 : Demonstration of method of preparation and interpretation of Bubble Stability Test.

1. Presence and severity of retraction in the upper chest.
2. Presence and severity of retraction of lower chest.
3. Presence and severity of retraction in xiphoid region.
4. Ni-latation of nares.
5. Presence of expiratory grunt.

Absence of features give the score of 0. When the sign is present in mild degree one, when present in marked degree two, total score of 0 indicates no RDS, score of 10 indicates RDS.

Method of Bubble Stability Test :

The bubble stability test was performed according to the method described by Clements and his co-workers (1972).

Requirements for the test :

1. Test tubes with an internal diameter of 10-14 mm.
2. 0.9% saline.
3. Ethanol 95%.
4. Amniotic fluid.

Method :

Before analysis, the tube containing the amniotic fluid was gently inverted several times to obtain a uniform suspension of particles. Volumes of 1.0 ml, 0.75 ml, 0.5 ml and 0.35 ml of amniotic fluid were pipetted and labelled as tube 1 - 1 : 1 dilution,

tube 2 - 1 : 1.3 dilution, tube 3 - 1 : 2 dilution, tube 4 - 1 : 4 dilution respectively. Volumes of 0.25, 0.50, 0.75 ml of 0.9% saline was added to the tubes 2, 3 and 4 respectively. Now 1 ml of 95% ethanol is added to each tube.

The tubes were capped with clean rubber stoppers and shaken vigourously for 15 seconds and then placed on a rack vertically. After 15 minutes, the tubes were viewed against a flat black background. A tube was labelled as positive if it showed a complete ring of bubbles in the meniscus. The tube with highest dilution of liquor giving such a result was noted.

The samples were interpreted as negative if the tube with dilution 1 : 1 is negative, intermediate if the tube with dilution 1 : 1.3 is positive and positive if the tube with dilution 1 : 2 or more is positive.

<u>Precautions while performing Bubble stability Test</u> :

Although this test is relatively simple but the technique is extremely important and there are a number of factors deserve careful consideration.

1. Glass tubes must be cleaned without remenant of soap, serum or biological fluid which might produce foam.

2. Diameter of tube should not be large as it may affect the foam stability by changing surface area of glass in contact with foam.

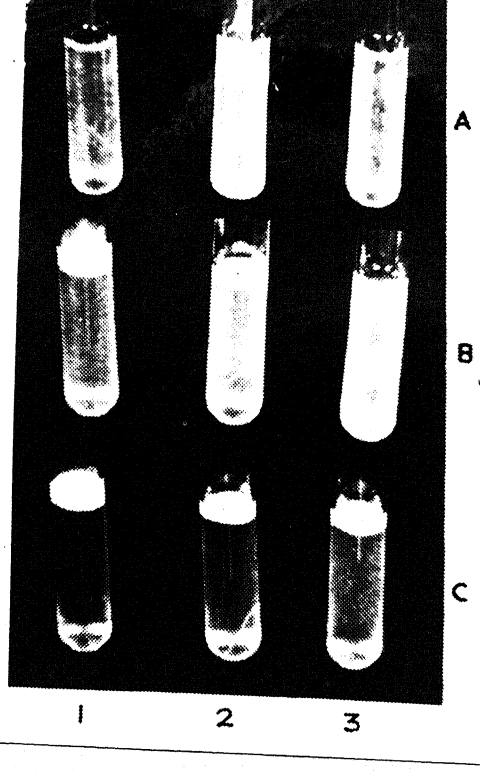

Fig. 3

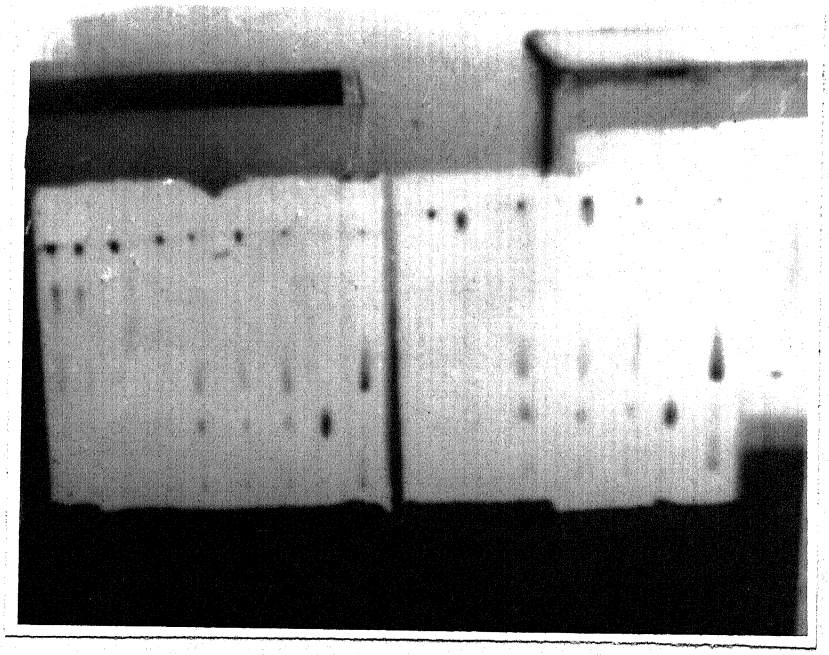

Fig. 4

Fig. 3 : Demonstration of Bubble Stability Test
(A) Negative, (B) Intermediate,
(C) Positive Test.
Test tubes (1,2,3) showing various
dilutions of liquor with normal saline.

Fig. 4 : Lecithin and sphingomyelin spots on
T.L.C. plate.

3. The tube should not be disturbed after the foam is produced to prevent breaking of the bubbles.

4. Amniotic fluid sample containing blood, meconium or vaginal secretion should not be used for shake test.

5. If the readings are missed, the tubes should not be re-shaken, but a new sample of AF to be used.

Lecithin/Sphingomyelin Ratio Determination :

Phospholipid extraction from amniotic fluid was done by modified method of Gluck et al (1971) and L/S ratio determined by planimetric method (Whitfield et al, 1972 and Gluck et al, 1974).

Reagents And Chemicals :

Standard Lecithin and spingomyelin : were obtained from V.P. Chest Institute, New Delhi and kept at -20°C.

Chemicals : All reagents were of analytic grade (A-R) or guaranteed reagents (G-R).

1. Silica gel - G
2. Chloroform
3. Methanol.
4. Normal saline (0.9%)
5. Acetic acid
6. Distilled water.

Extraction of Phospholipids from Amniotic Fluid :

Modified method of Gluck et al (1971) was used and L/S ratio was measured by planimetry method. 5 ml of clear fresh amniotic fluid or that stored at -20°c pipetted out in a separating funnel and mixed with equal volume of methanol (5 ml) and 2 volumes of chloroform (10 ml). This mixture was mixed and kept for 4 hours with intermittent shaking.

The lower layer, containing phospholipids in chloroform, was separated in a beaker and supernatant again extracted with equal volume of 2 : 1 chloroform-methanol mixture. To extract most of the phospholipids this process was repeated thrice.

Now all these separated samples were mixed with an equal volume of normal saline (0.9%) in a separating funnel and kept for 4-6 hours. So as to separate proteins and other sediments which precipitate as a slumy layer in between the two solutions.

The lower chloroform layer containing phospholipids was drawn into a beaker very carefully without disturbing the intermediate slumy layer. This solution was evaporated to dryness on water bath. For separation of individual phospholipids, the total extracted phospholipids were dissolved in known amount of chloroform (1 ml).

Thin Layer Chromatography (T.L.C.)

Preparation of thin layer chromatography plate :

Standard size plate in TLC was 20 x 20 cm transparent glass plate of 5 mm thickness. The plates should be cleaned with soap or non-abrasive detergent and finally rinse with distilled water followed by vertical draining and drying. Before coating with gel the plates were cleaned with cotton soaked in acetone to remove any trace of lipoidal material.

Now 30 gm. silica gel mixed with 100 ml of distilled water containing 0.05 M $NaHCO_3$ in a conical flask and shaken briskly for 30 seconds. In order to get a satisfactory plate the slurry must be spread evenly and immediately over the whole plate surface, as binder hydrates and sets within 2-3 minutes.

Slurry is best spread with one of commercially available T.L.C. applicator containing spreader sealer and leveler. The applicator was fixed at 0.25 mm thickness and slurry fed into the spreader which was drawn along set of plates in a single smooth motion.

On completion of coating, the plates were left as such at room temperature for overnight for layer to set. In general layers of 0.25 - 0.3 mm thickness were used (thickness of gel coating over glass plates).

Activation of Plates :

Activation involved drying the plates at an elevated temperature usually 110-130°C for 1-2 hours so as to remove chemically bounded water. Active plates usually pick up water rapidly from the atmosphere and thus the degree of activity changes so activated plate was usually reactivated immediately before use by further heating for 30 minutes at 110° C.

Application of Sample on TLC Plate :

The sample applied by micropipette. Use of spot-applicator plate ensure evenly spared spots 1 cm apart and 2 cm up from the edge. 0.05 ml of extracted phospholipids from each amniotic fluid sample was applied with intermittent drying so that spot area did not exceeds more than a few mm. in diameter.

Standard lecithin and sphingomyelin were marked by needle or pencil in front of the spot at the top of the plate with amount of quantity used. Lecithin and sphingomyelin spots of amniotic fluid phospholipids were identified by comparing the relative values with standard lecithin and sphingomyelin running on the same chromatograms.

Development of TLC Plates :

Brown glass tank with upward blowing was used so that the vertical plates will stand at an angle to the horizontal. Solvent surface should be erect and solvent

solution calm and quiet. Solvent used was chloroform : methanol : Acetic acid : distilled water, in ratio of 25 : 15 : 4 : 1.

For the two plate tank as in the present study 90 ml solvent was used. The solvent was made to run upto 17 cm height on the plate from base which used to take about 2-3 hours.

The plate was taken out and left to dry for 30 minutes at room temperature with a fast draught till no smell of solvent remained.

Visualisation of Spots :

The dry chromatogram was placed in a dry tank containing crystals of iodine which rapidly volatilise to purple vapours. A tank kept permanently for this purpose.

Lipid compounds absorb iodine reversibly to produce brown spots on a faint yellow background.

L/S ratio determination by planimetry method :

The amount of phospholipid on a chromatogram assessed by planimetry. The product of the length and width of individual spots provides a spot area measurement. The ratio of these values for lecithin and sphingomyelin being referred to as the L-S spot area ratio (LSAR).

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OBSERVATIONS

OBSERVATIONS

Bubble stability test and L/S ratio determination were done in a series of 210 cases both normal and abnormal pregnancies. These cases were divided into following two groups for the purpose of present study.

TABLE I

Distribution of cases according to type of cases.

Group	Type of cases	No.of cases	%
I	Cases of Normal pregnancy (control)	100	47.62
II	Cases of Abnormal pregnancy	110	52.38
Total		210	100.00

All the cases belonged to different age groups from 15 to 42 years, as evident from Table II.

TABLE II

Distribution of cases according to age.

Age-group (year)	Group I		Group II	
	No.of cases	%	No.of cases	%
15 - 19	22	22.00	34	30.91
20 - 24	39	39.00	30	27.28
25 - 29	24	24.00	22	20.00
30 - 34	9	9.00	12	10.91
35 - 39	4	4.00	8	7.27
7/ 40	2	2.00	4	3.63
Total	100	100.00	110	100.00

Table III shows the distribution of cases according to gravidity of the patients. The cases in the present study were varying from primigravida to tenth gravida. In group I 34 cases (34.00%) were primigravida while 60 cases (60.00%) were multigravida. But in group II 32.73% cases were primigravida and 60% were multigravida. The cases, who were gravida six or more were considered as grand multipara and were 6% and 7.27% in group I and II respectively.

TABLE XII

Distribution of cases according to gravidity.

Gravidity	Group I		Group II	
	No.of cases	%	No.of cases	%
Primigravida	34	34.00	36	32.73
Multigravida	60	60.00	66	60.00
Grand multipara	6	6.00	8	7.27
Total	100	100.00	110	100.00

In table IV, the cases were distributed according to the method of obtaining amniotic fluid (AF).

TABLE IV

Distribution of cases according to route of amniocentesis.

Route of amniocentesis	No.of cases	%
Per abdomen	76	36.20
Per vaginum	84	40.00
During caesarean section	42	20.00
During hysterotomy	8	3.80
Total	210	100.00

TABLE V

Distribution of cases according to period of gestation.

Period of gestation (weeks)	Group I		Group II	
	No.of cases	%	No.of cases	%
∠ 24	3	3.00	-	-
25 - 26	3	3.00	-	-
27 - 28	6	6.00	-	-
29 - 30	6	6.00	-	-
31 - 32	8	8.00	4	3.63
33 - 34	14	14.00	16	14.54
35 - 36	15	15.00	36	32.72
37 - 38	25	25.00	31	28.19
39 - 40	20	20.00	9	8.18
41 - 42	-	-	8	7.28
7/ 43	-	-	6	5.46
Total	100	100.00	110	100.00

Table V shows that maximum cases (25.00%) in group I are of 37-38 weeks of gestation while in group II maximum cases 36 (32.72%) were of 35-36 weeks gestation.

TABLE VI

Distribution of cases according to mode of delivery.

Mode of delivery	Group I		Group II	
	No.of cases	%	No.of cases	%
Vaginal	74	90.24	76	69.09
Caesarean section	8	9.76	34	30.91
Total	82	100.00	110	100.00

Of the total 82 cases (32-40 weeks gestation) 90.24% delivered vaginally and caesarean section was done in 9.76% in group I. In group of complicated pregnancy, 69.09% delivered vaginally while 30.91% had caesarean section as evident from table VI.

Table VII shows the results of Bubble stability test (BST) in 100 cases of normal pregnancy at various weeks of gestation (24-40 weeks). Cent per cent (100%) negative BST results were obtained from 24-30 weeks of gestation, while variable results (negative/intermediate/positive) were found from 31-40 weeks of gestation. At 33-34 weeks 50.00% cases were having positive BST result while at 39-40 weeks of gestation positive BST results were 85.00% (Graph No. 1).

Graph No-1

RESULTS OF BUBBLE STABILTY TEST IN RELATION TO PERIOD OF GESTATION (Graph No 9 in group I.)

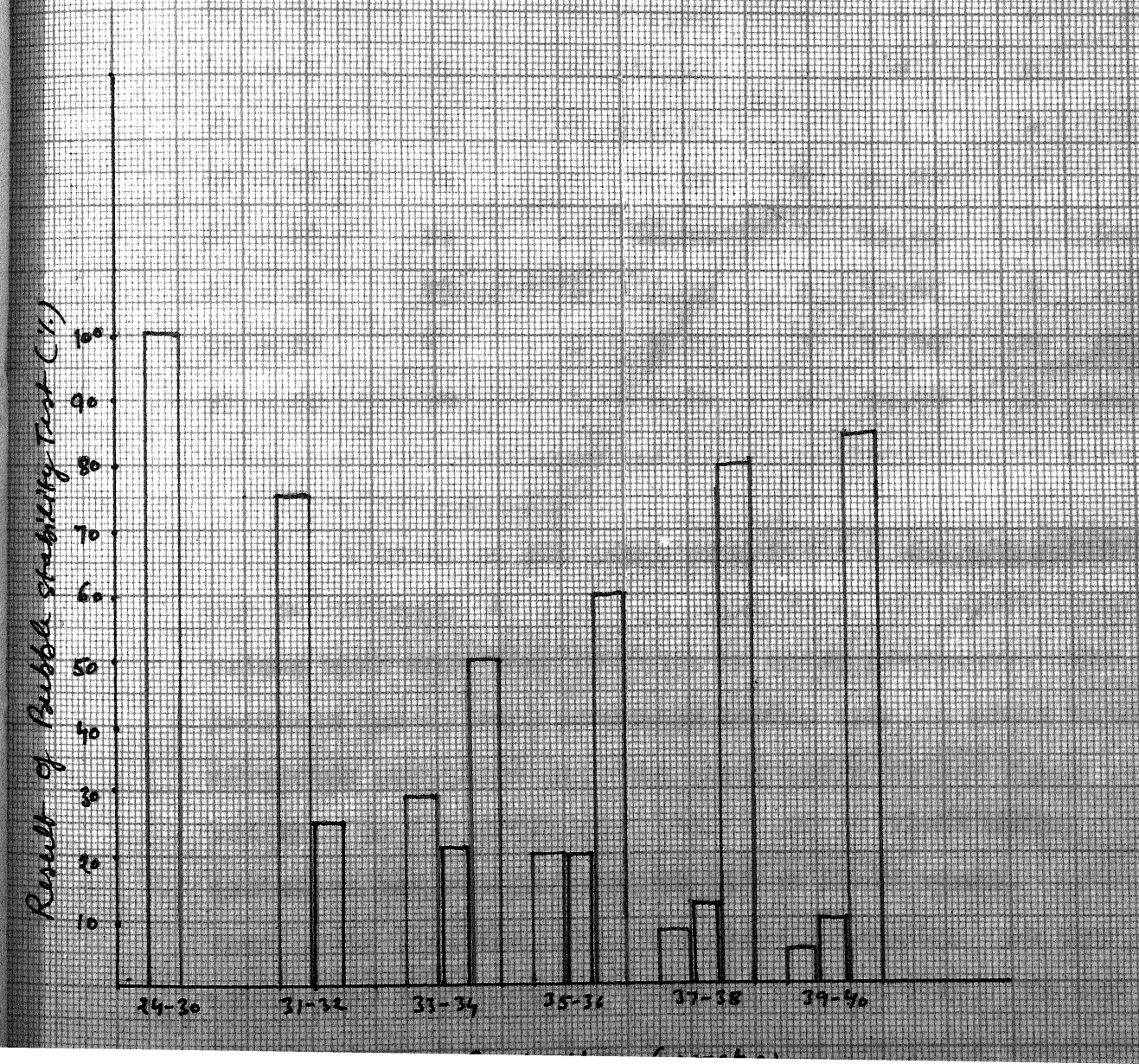


TABLE VII

Results of Bubble Stability Test (BST) in relation to period of gestation in group I.

Period of gestation (weeks)	Total No.of cases	Result of BST					
		Negative		Intermediate		Positive	
		No.	%	No.	%	No.	%
≤ 24	3	3	100.00	-	-	-	-
25 - 26	3	3	100.00	-	-	-	-
27 - 28	6	6	100.00	-	-	-	-
29 - 30	6	6	100.00	-	-	-	-
31 - 32	8	6	75.00	2	25.00	-	-
33 - 34	14	4	28.56	3	21.44	7	50.00
35 - 36	15	3	20.00	3	20.00	9	60.00
37 - 38	25	2	8.00	3	12.00	20	80.00
39 - 40	20	1	5.00	2	10.00	17	85.00

A total of 100 cases were studied and L/S ratio was determined. As shown in table VIII, the sphingomyelin values were more than lecithin in early pregnancy. The lecithin levels were gradually rising upto 34 weeks with advancing gestation and then there was a sudden surge of lecithin as its level was found 31.00 at 33-34 weeks gestation and increased to 53.526 at 35-36 weeks. After that again lecithin level increased gradually. On the other hand sphingomyelin showed a gradual fall. It was 55.625 at 24 weeks and at 39-40 weeks gestation decreased

Graph No. 2

MEAN VALUES OF LECITHIN AND SPHINGOMYELIN AT VARIOUS WEEKS OF GESTATION.
(in group I)

Lecithin —
Sphingomyelin —

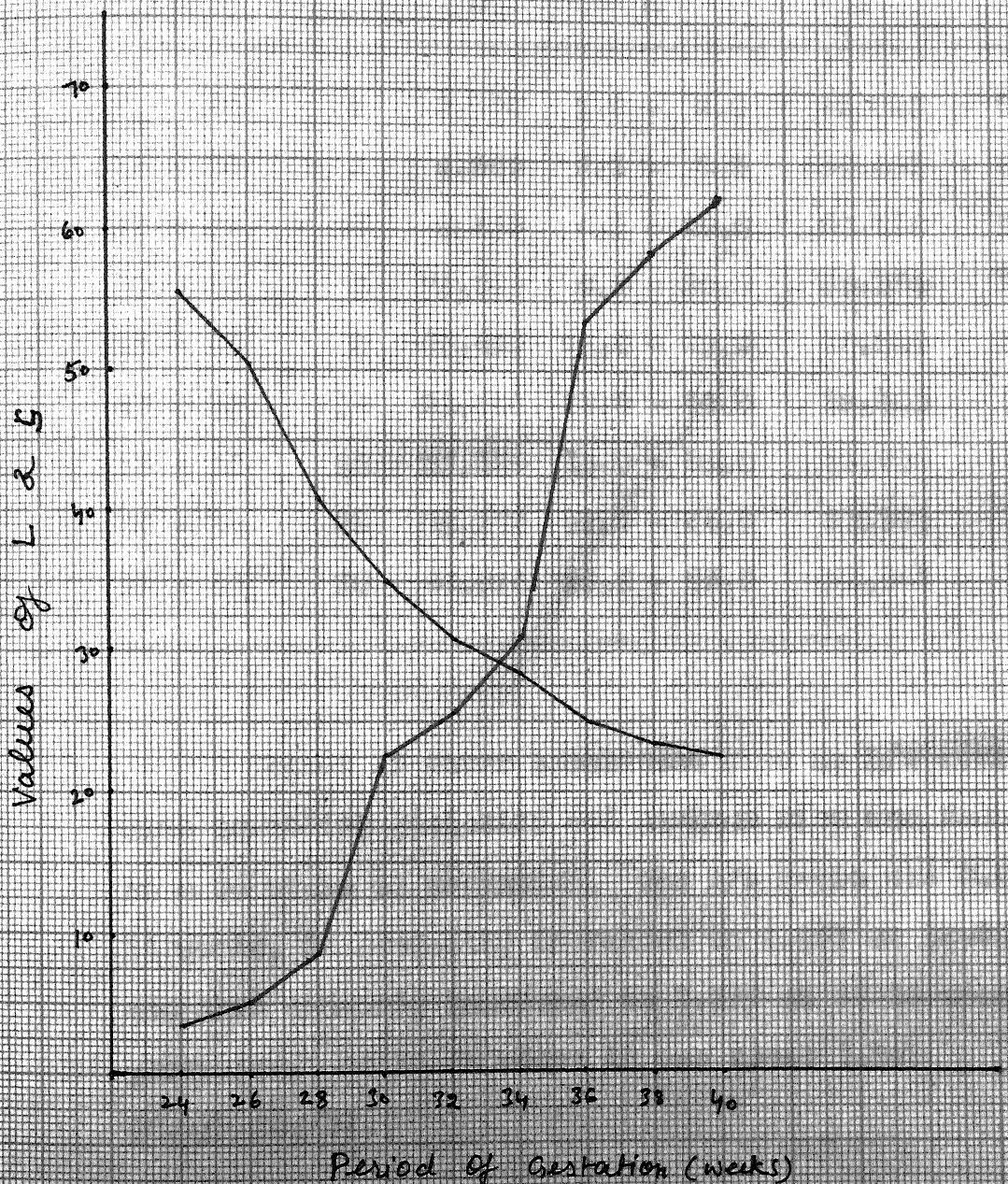


TABLE VIII

Lecithin (L) and sphingomyelin (S) levels in relation to period of gestation in group I.

Period of gestation (weeks)	Total No.of cases	Mean L	Range L	Mean S	Range S
∠ 24	3	2.900	1.0 - 4.8	55.625	50 - 62
25 - 26	3	4.500	2.5 - 5.5	50.635	45 - 56
27 - 28	6	8.620	4.5 - 16.0	40.850	36 - 63
29 - 30	6	22.645	8.0 - 28.0	35.250	30 - 48
31 - 32	8	25.482	14.0 - 40.0	30.850	22 - 44
33 - 34	14	31.000	22.0 - 44.0	28.500	16 - 38
35 - 36	15	53.526	28.0 - 58.0	25.000	22 - 32
37 - 38	25	58.543	32.0 - 64.0	23.520	15 - 32
39 - 40	20	62.814	30.0 - 66.0	22.543	14 - 30

Table IX shows a constant rise in L/S ratio with advancing gestation and maximum rise was found at 35 - 36 weeks of gestation. The L/S ratio was 0.054 at 24 weeks, it gradually increased to 1.165 at 33-34 weeks and then suddenly increased to 2.175 at 35-36 weeks gestation. At 39-40 weeks it was found 3.065 (Graph No. 3).

Graph No. 3

L/S RATIO VALUES AT VARIOUS WEEKS OF GESTATION IN GROUP I.

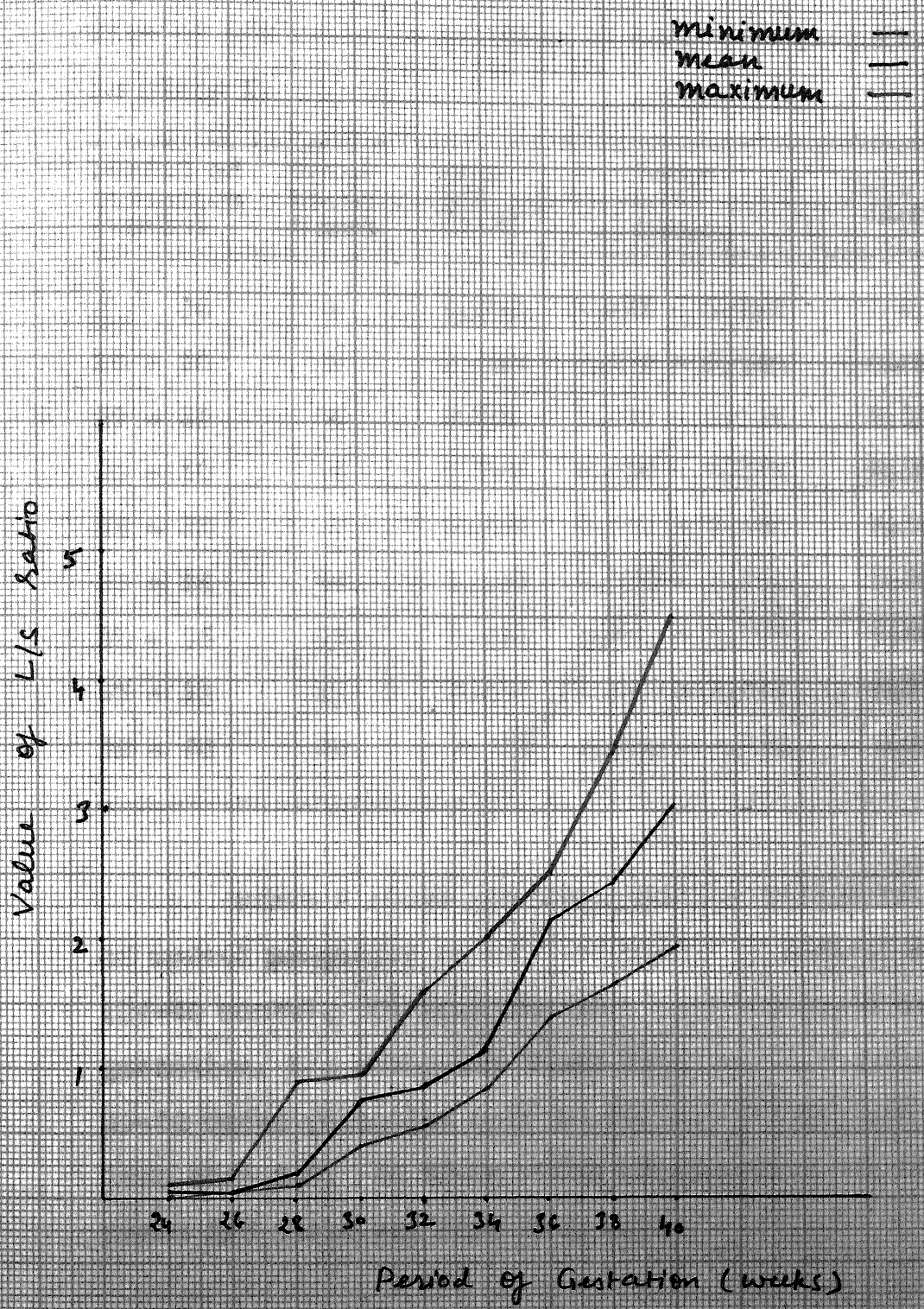


TABLE IX

Rise of L/S ratio in relation to period of gestation in group I.

Period of gestation (weeks)	Total No.of cases	Mean L/S ratio	Range L/S ratio	Rate of rise
≤ 24	3	0.054	0.018 - 0.088	-
25 - 26	3	0.088	0.058 - 0.140	0.034
27 - 28	6	0.209	0.084 - 0.964	0.121
29 - 30	6	0.748	0.420 - 0.924	0.539
31 - 32	8	0.866	0.532 - 1.620	0.118
33 - 34	14	1.165	0.850 - 2.000	0.299
35 - 36	15	2.175	1.400 - 2.900	1.010
37 - 38	25	2.485	1.640 - 3.455	0.310
39 - 40	20	3.065	1.950 - 4.550	0.580

Table X shows L/S ratio results in 100 cases of normal pregnancy at various period of gestation (24-40 weeks). In cent per cent cases of 24-30 weeks gestation, L/S ratio was found less than 2.0 while at 39-40 weeks gestation only 10.00% were having L/S ratio less than 2.0. At 35-36 weeks of gestation, in 66.67% cases L/S ratio was more than 2.0. (what about between 30 - 35 wks).

TABLE X

Distribution of L/S ratio at various period of gestation in group I.

Period of gestation (weeks)	Total No.of cases	L/S ratio					Percentage	
		0.5	0.6-1.0	1.1-1.5	1.6-2.0	2.0	<2.0%	2.0%
< 24	3	3	-	-	-	-	100.0	-
25 - 26	3	3	-	-	-	-	100.0	-
27 - 28	6	4	2	-	-	-	100.0	-
29 - 30	6	2	4	-	-	-	100.0	-
31 - 32	8	1	2	2	2	1	87.50	12.50
33 - 34	14	-	2	4	6	2	85.72	14.28
35 - 36	15	-	-	2	3	10	33.33	66.67
37 - 38	25	-	-	1	3	21	16.00	84.00
39 - 40	20	-	-	-	2	18	10.00	90.00

Table XI shows the correlation of results of bubble stability test (BST) and L/S ratio with respiratory status of live born from 32-40 weeks of gestation. In 16 cases with RDS, 14 had negative BST result, 2 had intermediate BST result and L/S ratio less than 1.9. Among 59 cases who had no respiratory distress, 55 cases were having L/S ratio two or more than two while BST results were positive in 53 cases (Graph No. 4).

TABLE XI

Correlation of bubble stability test (BST) and L/S ratio with respiratory status of live-born infant (32-40 weeks) in group I.

Result of BST	Total No.of cases	Clinical result					
		RDS		TRD		No RD	
		No.	%	No.	%	No.	%
Negative	16	14	87.50	2	12.50	-	-
Intermediate	13	2	15.38	5	38.46	6	46.16
Positive	53	-	-	-	-	53	100.00
Total	82	16	19.51	7	8.53	59	71.96
L/S ratio							
$\angle$ 1.5	12	12	100.00	-	-	-	-
1.5 - 1.9	15	4	26.67	7	41.66	4	26.67
$\nearrow$ 2.0	55	-	-	-	-	55	100.00
Total	82	16	19.51	7	8.53	59	71.96

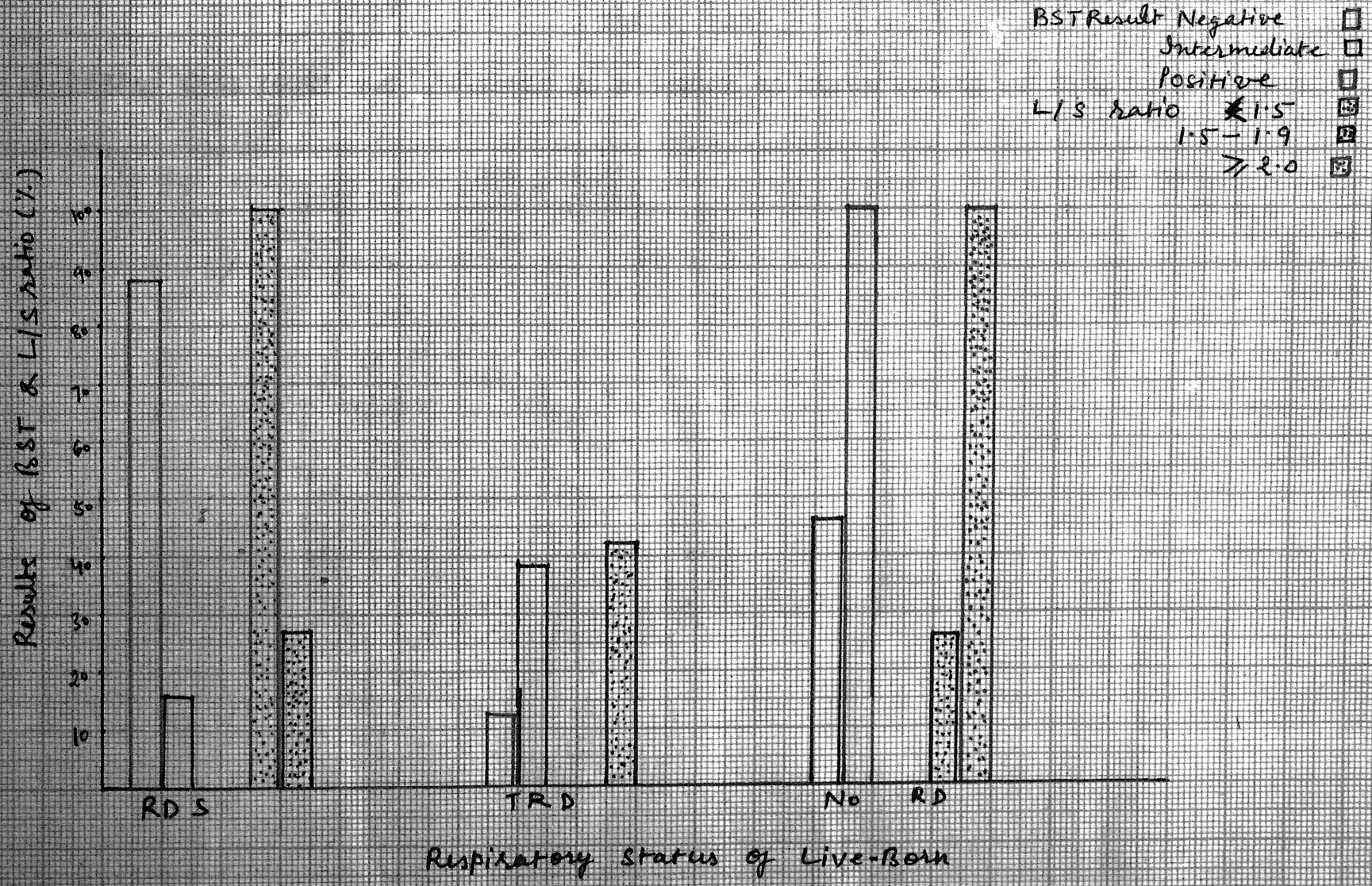
RDS = Respiratory distress syndrome.

TRD = Transient respiratory distress.

RD = Respiratory distress.

Graph No. 4.

BUBBLE STABILITY TEST AND L/S RATIO IN RELATION TO RESPIRATORY STATUS OF LIVE-BORN.



RDS = Respiratory Distress Syndrome, TRD = Transient Respiratory Distress, No RD = No Respiratory Distress

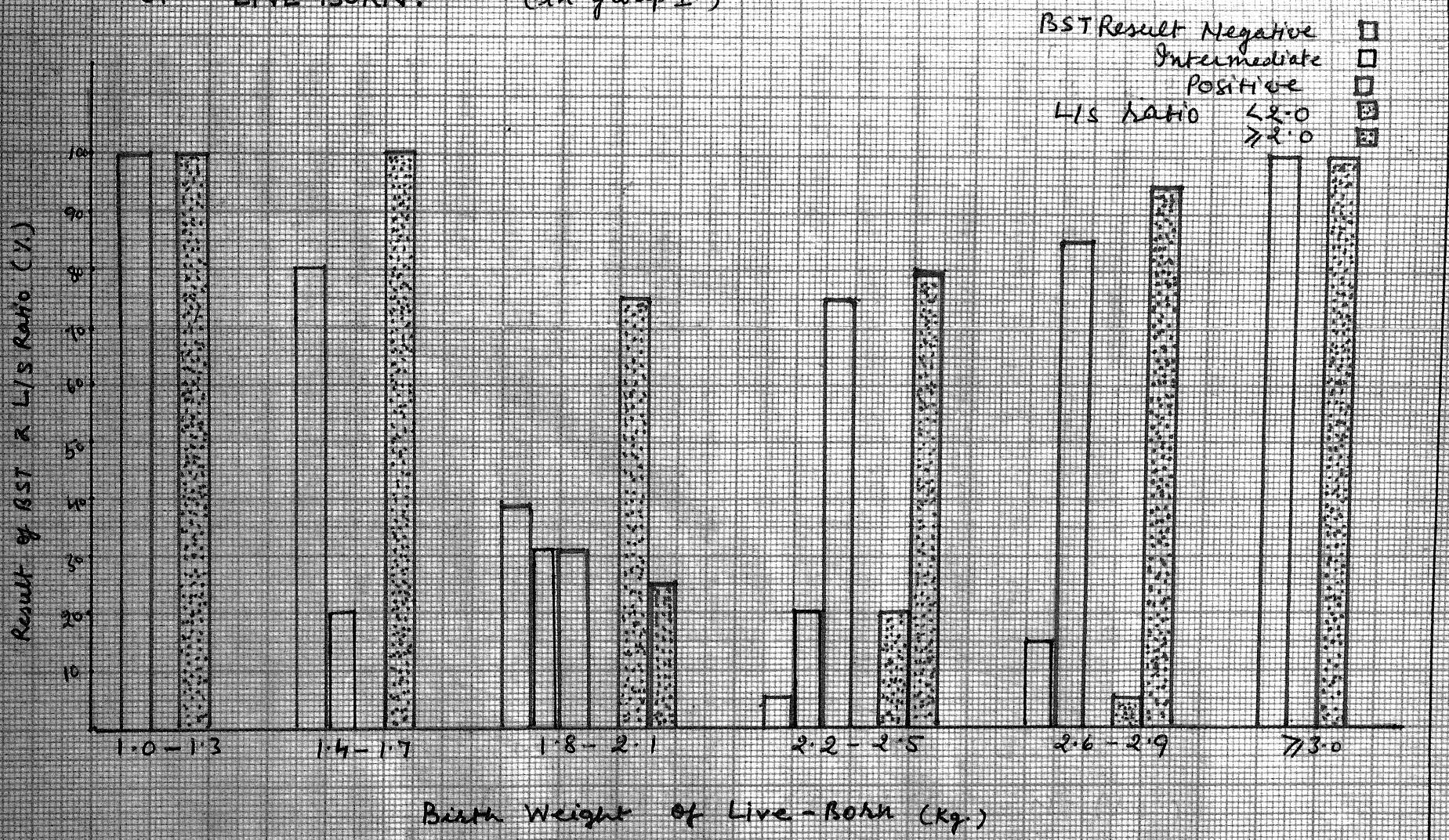
TABLE XII

Bubble stability test (BST) and L/S ratio in relation to birth weight of live born in group I.

Weight (kg.)	Total No.of cases	BST result						L/S ratio			
		Negative		Intermediate		Positive		< 2.0		≥ 2.0	
		No.	%	No.	%	No.	%	No.	%	No.	%
1.0 - 1.3	5	5	100.00	-	-	-	-	5	100.00	-	-
1.4 - 1.7	5	4	80.00	1	20.00	-	-	5	100.00	-	-
1.8 - 2.1	16	6	37.50	5	31.25	5	31.25	12	75.00	4	25.00
2.2 - 2.5	20	1	5.00	4	20.00	15	75.00	4	20.00	16	80.00
2.6 - 2.9	20	-	-	3	15.00	17	85.00	1	5.00	19	95.00
≥ 3.0	16	-	-	-	-	16	100.00	-	-	16	100.00

Graph No. 5

BUBBLE STABILITY TEST AND L/S RATIO IN RELATION TO BIRTH-WEIGHT OF LIVE-BORN. (in group I.)



Marked correlation was observed between L/S ratio and bubble stability test (BST) with birth weight of live born in a series of 82 cases of normal pregnancy ranging from 32-40 weeks of gestation. As evident from table XXI, all babies weighing less than 1.7 kg had L/S ratio less than 2.0 and 90 per cent were having BST result negative, while 10 per cent were with intermediate result. Neonates having birth weight 2.2 to 3.0 kg. or more showed positivity of BST varying from 75 - 100 per cent and L/S ratio more than 2.0 in 80-100 per cent cases (Graph No. 5).

Table XXII shows the relationship of bubble stability test (BST) with Apgar score in group I. Before 34 weeks of gestation 95.45% had Apgar score less than seven while after 34 weeks of gestation, in 60% Apgar score more than seven. L/S ratio was found less than 2.0 in 100 per cent cases with Apgar score 1-4, irrespective of period of gestation. In 100 per cent cases L/S ratio was more than 2.0 where babies having Apgar score 8-10. Only one (4.55%) baby was found with Apgar score of 8-10 before 34 weeks of gestation and 18.18% were having positive BST result and L/S ratio more than 2.0 in 22.72% cases. It is also evident from table XXII (Graph No. 6).

TABLE XIII

Bubble stability test (BST) and L/S ratio in relation to Apgar score in group I.

Period of gestation	Apgar score	Total No. of cases	BST results						L/S ratio			
			Negative		Intermediate		Positive		< 2.0		> 2.0	
			No.	%	No.	%	No.	%	No.	%	No.	%
Before 34 weeks	1 - 4	6	3	50.00	3	50.00	-	-	6	100.00	-	-
	5 - 7	15	10	66.67	2	13.33	3	20.00	11	73.33	4	26.67
	8 - 10	1	-	-	-	-	1	100.00	-	-	1	100.00
After 34 weeks	1 - 4	2	2	100.00	-	-	-	-	2	100.00	-	-
	5 - 7	10	4	40.00	2	20.00	4	40.00	6	60.00	4	40.00
	8 - 10	48	-	-	6	12.50	42	87.50	-	-	48	100.00

Graph No. 6

BUBBLE STABILITY TEST AND L/S RATIO IN RELATION TO APGAR SCORE. (in group I)

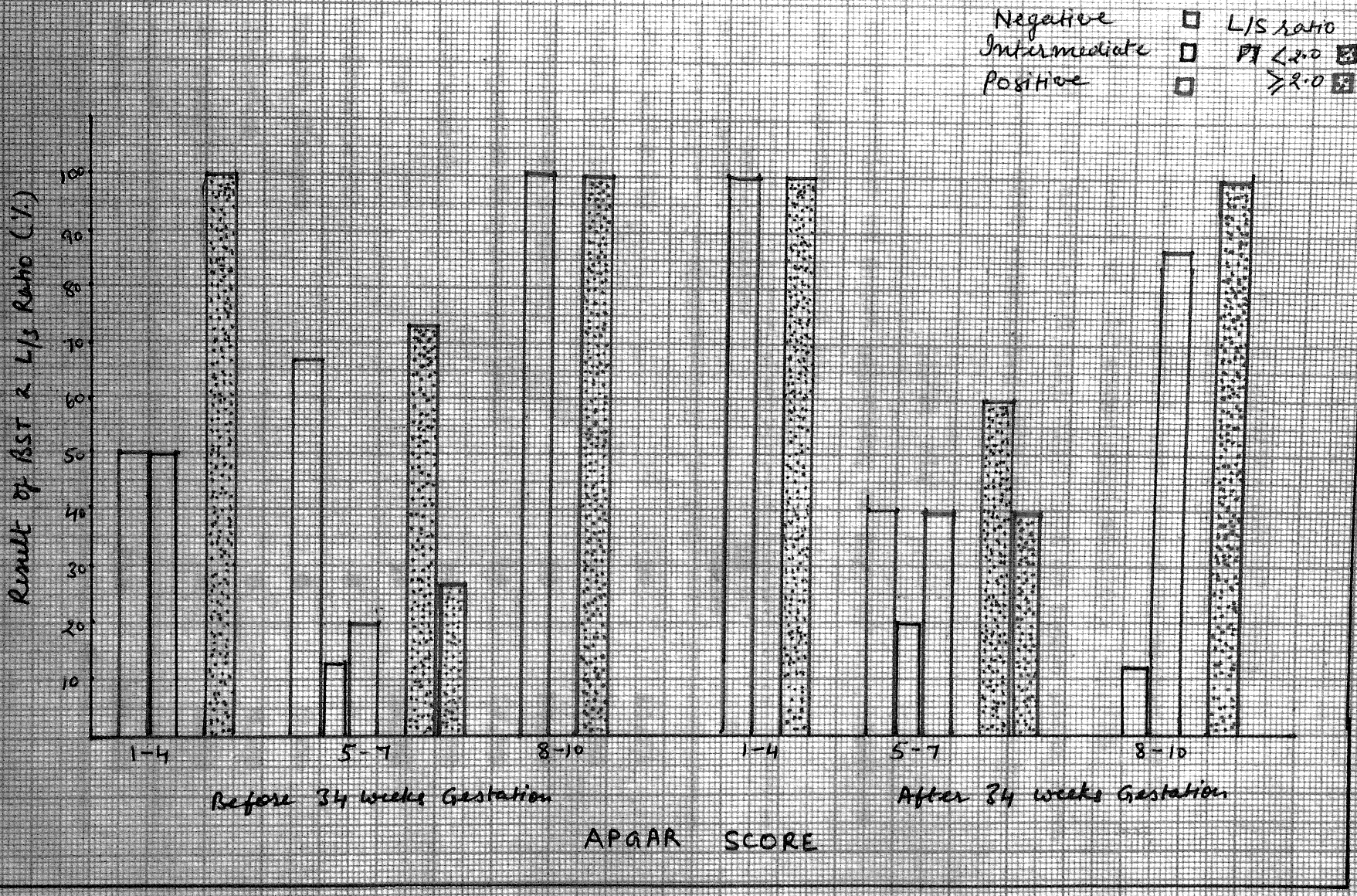


Table XIV shows distribution of cases according to type of complicated pregnancy in group II, including various abnormalities either in mother or in fetus. A total of 110 cases were studied. 52.72% cases were associated with affection of fetus while rest of 47.28% were associated with complications of mother.

TABLE XIV

Distribution of cases according to type of complication of pregnancy in group II.

Type of complication of pregnancy.	No.of cases	%
Post maturity	14	12.72
Fetal distress	18	16.36
Toxaemia of pregnancy	20	18.19
Antepartum haemorrhage (APH)	16	14.55
Hydramnios	10	9.10
Twins	14	12.72
Hydrocephalus	2	1.81
Heart disease	6	5.46
Diabetes	6	5.46
Rh immunisation	4	3.63
Total	110	100.00

Table XV shows the result of bubble stability test (BST) and L/S ratio in various complicated pregnancies.

TABLE XV

Results of bubble stability test (BST) and L/S ratio in relation to period of gestation in group

Type of complication	Period of gestation (weeks)	Total No. of cases	BST results: Negative No.	Negative %	Intermediate No.	Intermediate %	Positive No.	Positive %	L/S ratio: Mean	L/S ratio: Range
Post Maturity	41 - 42	8	-	-	-	-	8	100.00	3.63	3.44 - 4.12
	43 & more	6	-	-	-	-	6	100.00	3.83	3.55 - 4.15
Fetal Distress	33 - 34	4	1	25.00	2	50.00	1	25.00	1.837	1.77 - 1.904
	35 - 36	4	1	25.00	1	25.00	2	50.00	2.150	1.90 - 2.26
	37 - 38	5	1	20.00	1	20.00	3	60.00	2.246	1.70 - 2.34
	39 - 40	5	-	-	-	-	5	100.00	2.850	1.95 - 3.65
Twins	31 - 32	2	2	100.00	-	-	-	-	1.00	-
	33 - 34	4	2	50.00	2	50.00	-	-	1.45	1.00 - 1.80
	35 - 36	4	-	-	2	50.00	2	50.00	2.22	1.75 - 2.70
	37 - 38	4	-	-	2	50.00	2	50.00	2.40	2.00 - 2.80
Rh-immunization	35 - 36	1	-	-	1	100.00	-	-	1.90	-
	37 - 38	2	-	-	1	50.00	1	50.00	2.24	1.94 - 2.54
	39 - 40	1	-	-	-	-	1	100.00	2.50	-

Contd.....

TABLE XV (Contd...)

Type of complication	Period of gestation (weeks)	Total No.of cases	BSF result						L/S ratio	
			Negative		Intermediate		Positive		Mean	Range
			No.	%	No.	%	No.	%		
Hydrocephalus	35 - 36	1	1	100.00	-	-	-	-	1.7	-
	37 - 38	1	-	-	1	100.00	-	-	1.9	-
Hydramnios	35 - 36	4	2	50.00	2	50.00	-	-	1.9	1.76 - 2.2
	37 - 38	6	4	66.67	2	33.33	-	-	2.2	1.8 - 2.4
Heart Disease	35 - 36	4	-	-	1	25.00	3	75.00	2.10	1.9 - 2.4
	37 - 38	2	-	-	-	-	2	100.00	2.3	1.9 - 2.6
Diabetes	35 - 36	2	-	-	1	50.00	1	50.00	2.4	2.3 - 2.6
	37 - 38	3	-	-	1	33.33	2	66.67	3.4	2.9 - 3.6
	39 - 40	1	-	-	-	-	1	100.00	3.2	-
APH	31 - 32	2	2	100.00	-	-	-	-	1.00	-
	33 - 34	4	1	25.00	2	50.00	1	25.00	1.88	1.5 - 2.0
	35 - 36	6	1	16.67	2	33.33	3	50.00	2.38	1.8 - 2.6
	37 - 38	4	-	-	1	25.00	3	75.00	2.75	2.0 - 3.6
Toxaemia of Pregnancy	33 - 34	4	3	75.00	1	25.00	-	-	2.00	1.0 - 2.2
	35 - 36	10	2	20.00	4	40.00	4	40.00	2.2	1.2 - 2.4
	37 - 38	4	-	-	1	25.00	3	75.00	2.4	1.4 - 3.8
	39 - 40	2	-	-	1	50.00	1	50.00	2.8	2.6 - 3.0

Post Maturity :

In 14 cases of post maturity 100% cases were having bubble stability test (BST) positive with a L/S ratio more than 3.0. None of the babies developed respiratory distress and all babies were having birth weight more than 2.5 kg as evident from table XV, XVI, XVII & XVIII, Graph No. 7 and 8.

Fetal Distress :

In a series of 18 cases of fetal distress varying from 33-40 weeks gestation, 44.44 per cent cases were having L/S ratio less than 2.0 and 66.66 per cent were with more than 2.0 L/S ratio. In 16.67% cases BST results were negative and in 61.11% were positive. 5 babies developed RDS, 5 developed TND and 8 babies were without any respiratory distress (Table XV, XVI, XVII & XVIII and Graph No. 7, 8).

Twins :

In 14 cases of twin pregnancy as shown in tables XV, XVI, XVII & XVIII. 4 (28.57%) had negative, 6 (42.86%) had intermediate and 4 (28.57%) had positive BST results. L/S ratio was found less than 2.0 in 64.29% cases. 5 babies developed RDS, 3 had TND and 6 babies were without any respiratory difficulties. 71.43% of the babies were having birth weight less than 2.0 kg.

Rh-immunization :

In the present study 4 cases of Rh-immunization were studied. 50% cases were found with L/S ratio more than 2.0 and positive BST result. Only one baby developed transient respiratory distress but recovered. Birth weight in 50% babies were more than 2.5 kg.

Hydrocephalus :

Two cases of hydrocephalus were studied. In one case intermediate BST result was found and L/S ratio 1.9 with 2.5 kg birth weight. Baby developed RDS. Other baby with L/S ratio 1.7 and negative BST result. delivered by needling to drain cerebro-spinal fluid (CSF) was still birth.

Hydramnios :

Ten cases of hydramnios were studied. 60% were having L/S ratio more than 2.0 and BST result was negative in 60% and intermediate in 40%. None of the case was having positive BST result. One baby was still birth. No baby developed RDS except one who had transient respiratory distress and recovered. 20% babies were having birth weight less than 2.0 kg (Tables XV, XVI, XVII & XVIII).

Heart Disease :

Among 6 cases one new born developed RDS who was having L/S ratio less than 2.0 and BST result was intermediate. Rest of the babies were without any respiratory difficulties. 50% babies were having birth weight 2.5 kg (Table XV, XVI, XVII, XVIII).

Diabetes :

In 6 cases of diabetes as shown in table XV, XVI, XVII, XVIII, cent per cent cases were having L/S ratio more than 2.0 and BST result positive in 66.67%. Only 2 babies developed transient respiratory distress but recovered. In 83.33% cases birth weight of new born was more than 2.5 kg.

APH :

In a series of 16 cases of antepartum haemorrhage (APH) varying from 31-38 weeks of gestation, L/S ratio was less than 2.0 in 50% and BST was positive in 43.75%. Two babies were still birth, 3 developed RDS and 2 developed transient respiratory distress. Only 25% babies were having birth weight more than 2.5 kg. (Tables XV, XVI, XVII & XVIII).

TABLE XVI

Correlation of results of bubble stability test (BST) with respiratory status of live born in complicated pregnancies.

Type of complication	Total No.of cases	BST result: Negative No.	Negative %	Intermediate No.	Intermediate %	Positive No.	Positive %	Respiratory status
Post Maturity	14	-	-	-	-	14	100.00	No RD.
Fetal Distress	18	3	16.67	4	22.22	11	61.11	Negative 3 RDS. Intermediate 2 TRD, 2 RDS. Positive 3 TRD, 8 No RD.
Twins	14	4	28.57	6	42.86	4	28.57	Negative 3 RDS, 1 TRD. Intermediate 2 RDS, 2 TRD, 2 No RD. Positive 4 No RD.
Rh-immunisation	4	-	-	2	50.00	2	50.00	Intermediate 1 TRD, 1 No RD. Positive 2 No RD.
Hydrocephalus	2	1	50.00	1	50.00	-	-	1 still born, Intermediate 1 RDS.
Hydramnios	10	6	60.00	4	40.00	-	-	Negative 1 still born, 1 TRD, 4 No RD. Intermediate 4 No RD.
Heart Disease	6	-	-	1	16.66	5	83.34	Intermediate 1 RDS. Positive 1 TRD, 4 No RD.
Diabetes	6	-	-	2	33.33	4	66.67	Intermediate 2 TRD. Positive 4 No RD.
APH	16	4	25.00	5	31.25	7	43.75	Negative 2 still birth, 2 died dum to R Intermediate 1 RDS, 3 TRD, 1 No RD. Positive 7 No RD.
Toxaemia of Pregnancy.	20	5	25.00	7	35.00	8	40.00	Negative 1 still birth, 2 RDS, 2 TRD, Intermediate 2 RDS, 3 TRD, 1 No RD. 1 still birth. Positive 1 RDS, 7 No RD.

RDS = Respiratory distress syndrome; TRD=Transient respiratory distress; RD=Respiratory distress.

Graph No. 7

BUBBLE STABILITY TEST IN VARIOUS ABNORMAL PREGNANCIES.

TABLE XVII

Correlation of L/S ratio with respiratory status of live born in complicated pregnancies.

Type of complication	Total No.of cases	L/S ratio < 2.0 No.	< 2.0 %	L/S ratio ≥ 2.0 No.	≥ 2.0 %	Respiratory status
Post Maturity	14	-	-	14	100.00	No RD.
Fetal Distress	18	8	44.44	10	55.56	< 2.0 - 4 RDS, 4 TRD, ≥ 2.0 - 1 RDS, 1 TRD, 8 No RD.
Twins	14	9	64.28	5	35.72	< 2.0 - 6 RDS, 2 TRD, 3 No RD. ≥ 2.0 - 1 RDS, 1 TRD, 3 No RD.
Sh-immunization	4	2	50.00	2	50.00	< 2.0 - 1 TRD, 1 No RD. ≥ 2.0 - 2 No RD.
Hydrocephalus	2	2	100.00	-	-	1 still birth, 1 RDS.
Hydramnios	10	6	60.00	4	40.00	1 still birth, < 2.0 - 1 TRD, 4 No RD. ≥ 2.0 - 4 No RD.
Heart Disease	6	1	16.66	5	83.34	< 2.0 - 1 RDS, ≥ 2.0 - 5 No RD.
Diabetes	6	-	-	6	100.00	2 TRD, 4 No RD.
APH	16	8	50.00	8	50.00	< 2.0 - 2 still birth, 2 RDS, 2 TRD, 1RD ≥ 2.0 - 1 TRD, 7 No RD.
Toxaemia of pregnancy	20	10	50.00	10	50.00	< 2.0 - 3 still birth, 3 RDS, 4 TRD, ≥ 2.0 - 1 RDS, 1 TRD, 8 No RD.

RDS=Respiratory distress syndrome; TRD=Transient respiratory distress; RD=Respiratory distress.

Graph No. 8

L/S RATIO VALUES IN VARIOUS ABNORMAL PREGNANCIES.

TABLE XVIII

Bubble stability test (BST) and L/S ratio in relation to birth weight of live born infants in groupII.

Type of complication	Total No.of cases	Birth weight (kg.)						BST result						L/S ratio			
		< 2.0		2.0-2.5		> 2.5		Negative		Intermediate		Positive		< 2.0		> 2.0	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Post-Maturity	14	-	-	-	-	14	100.00	-	-	-	-	14	100.00	-	-	14	100.00
Fetal Distress	18	3	16.67	6	33.33	9	50.00	3	16.67	4	22.22	11	61.11	8	44.44	10	55.56
Twins	14	10	71.43	4	28.57	-	-	4	28.57	6	42.86	4	28.57	9	64.28	5	35.72
Rh-immunisation	4	-	-	2	50.00	2	50.00	-	-	2	50.00	2	50.00	2	50.00	2	50.00
Hydrocephalus	2	1	50.00	1	50.00	-	-	1	50.00	1	50.00	-	-	2	100.00	-	-
Hydramnios	10	2	20.00	6	60.00	2	20.00	6	60.00	4	40.00	-	-	6	60.00	4	40.00
Heart Disease	6	1	16.67	3	50.00	2	33.33	-	-	1	16.66	5	83.34	1	16.66	5	83.34
Diabetes	6	-	-	1	16.67	5	83.33	-	-	2	33.33	4	66.67	-	-	6	100.00
APH	16	5	31.25	7	43.75	4	25.00	4	25.00	5	31.25	7	43.75	8	50.00	8	50.00
Toxaemia of Pregnancy	20	3	15.00	9	45.00	8	40.00	5	25.00	7	35.00	8	40.00	10	50.00	10	50.00

Toxaemia of pregnancy :

In the present study, 20 cases of toxaemia of pregnancy varying from 32-40 weeks of gestation were studied. L/S ratio was more than 2.0 in 50% cases and BST was positive in 40.00%, intermediate in 35% and negative in 25% cases. Four babies developed RDS and 5 had transient respiratory distress. Three babies were still birth. 40% babies were having birth weight more than 2.5 kg (Tables XV, XVI, XVII & XVIII).

TABLE XIX

Comparative incidence of RDS in cases of normal and abnormal pregnancies.

Period of gestation	Type of pregnancy	Total No.of cases	No.of infants with RDS	RDS %
Before 34 weeks	Normal	22	10	45.45
	Abnormal	20	5	25.00
	Total	42	15	35.72
After 34 weeks	Normal	60	6	10.00
	Abnormal	90	14	15.55
	Total	150	20	13.33

Table XIX shows a comparative incidence of RDS in cases of normal and abnormal pregnancies before and after 34 weeks of gestation. Before 34 weeks, incidence of RDS was higher in normal pregnancy (45.45%), whereas after 34 weeks higher incidence of RDS has seen in abnormal pregnancy (15.55%). Over all incidence was found to be 13.33% after 34 weeks and 35.72% before 34 weeks gestation.

TABLE XX

Incidence of RDS according to route of delivery.

Route of delivery	Total No. of cases	No.of infants with RDS	RDS %
Vaginal	150	24	16.00
Caesarean	42	11	26.19
Total	192	35	18.13

Table XX shows significant difference in the incidence of RDS delivered vaginally or by caesarean section. Higher incidence of RDS was seen in cases delivered by caesarean section (26.19%).

Table XXI shows relationship of RDS to Apgar score. RDS was associated with low Apgar score irrespective of period of gestation.

TABLE XXX

Relation of RDS with Apgar score in cases before and after 34 weeks of gestation.

Period of gestation	Apgar score	Total No. of cases	No.of cases with RDS	RDS %
Before 34 weeks	1 - 4	14	10	71.42
	5 - 7	26	6	23.07
	8 - 10	2	-	-
After 34 weeks	1 - 4	8	6	75.00
	5 - 7	34	12	35.29
	8 - 10	78	2	2.56

Table XXIX shows that the risk of RDS was invariably related to birth weight. With birth weight more 3.0 the chances of developing RDS were nil.

TABLE XXIX

Incidence of RDS in relation to birth weight of live born.

Birth weight	Total No. of cases	No.of infants with RDS	RDS %
1.0 - 1.5	24	16	66.66
1.6 - 2.0	40	10	25.00
2.1 - 2.5	54	7	12.96
2.6 - 3.0	46	2	4.34
3.1 - 3.5	24	-	-
7/ 3.6	4	-	-

****

DISCUSSION

DISCUSSION

Respiratory function in the new born depends on the intra-uterine development of adequate amount of surfactant within the epithelial cells of the alveoli (Adams et al, 1965; Brumley et al, 1967). The surface active material is believed to be produced by type II epithelial cells of the alveoli. Since fluid passes from the fetal respiratory tract to the amniotic cavity carrying with its suspended particles of surfactant from the alveoli (Biggs et al, 1973), the concentration in fetal lung tissue should be reflected to the surfactant content of the amniotic fluid (Nelson, 1972). Hence the most important current need in assaying the fetus is a simple procedure to provide reliable information about the degree of pulmonary maturity. The present study of comparison of Bubble Stability Test (BST) and L/S ratio determination for fetal lung maturity was undertaken to ascertain whether such study would prove helpful in providing a simple reliable procedure.

Relationship between L/S Ratio, Bubble Stability Test and Gestational Age :

In our study, the presence of surfactant by bubble stability test could not be detected upto 30 weeks

of gestation (Table VII). Upto this period of gestation mean L/S ratio was found to be 0.748 (Table IX). From 31-32 weeks of gestation appearance of surfactant by bubble stability test was doubtful as no positive results were obtained (Table VII). Surfactant started appearing abruptly at about 33 weeks gestation (50%) and was positive in 85% of all cases over 38 weeks.

It was observed that bubble stability test was in linear correlation with gestational age. Positive result indicates a fetus of 34 weeks of gestation or more in 80% cases. Negative result suggests gestational age of less than 34 weeks in 90% cases. Intermediate results are not conclusive.

Clements et al (1972) observed that an appreciable titer of surfactant appeared on the average at 33 weeks estimated gestation, but the time of appearing surfactant is variable from 25 weeks to term. Gluck et al (1972) found biochemical maturation of lung at 35 weeks. Lemons and Jaffe (1973) have observed alveolar stability of lung by 34-36 weeks of gestation. Koun et al (1973) have found that positive BST result indicates gestation of 36 weeks or more, while negative and intermediate BST results indicate 35 weeks or less gestation. Reddy et al (1978) found appreciable titer of surfactant at 35 weeks. Shushan et al (1979) observed positive bubble stability test after 36 weeks gestation.

Donald and Calvin (1974) and Myers et al (1975) observed no correlation between bubble stability test and gestational age.

In our study lecithin was found to show constant rise throughout pregnancy but the maximum rise was observed between 35-36 weeks. Bhagwanani et al (1972) noted rising values of lecithin throughout pregnancy till term but the surge occurred at 34 weeks of gestation. Our findings are consistant with their observation (Table VIII).

Dunn and Shatnagar (1973) reported a gradual rise in each of these phospholipids beginning at 16th week and continuing as pregnancy approaching term.

Gluck et al (1971) saw a surge in lecithin concentration at 36th week of gestation which is in accordance with our findings, while Bhagwanani et al (1972) observed exaggeration from 34 weeks. Biesenski (1973) found a similar trend in lecithin concentration.

Reverse trend was observed with sphingomyelin values (Table VIII). A gradual fall was seen which was also seen by Arvidson et al (1972), Biesenski (1973). Both Gluck (1971) and Dunn et al (1973) have reported rising values of sphingomyelin throughout pregnancy which was opposite to our observation.

L/S ratio was found to show a steady rise with increasing gestational age and the critical point of 2.0 was reached at about 36th week (Table IX). The mean values of L/S ratio were quite low before 34 weeks i.e. less than 1.0 and between 25-26 weeks gestation was 0.088 as shown in table IX. Sharma et al (1981) have reported mean value 0.096 between 26-28 weeks almost in accordance to our values.

It is clear from table X that no values of L/S ratio exceeded 2.0 prior to 30th week of gestation. Till 34 weeks more cases (85%) had less than 2.0 ratio while between 35-36 weeks the pattern reversed, most of the cases (66.67%) had L/S ratio more than 2.0. In many published work the mature ratio has been reached earlier between 34th and 37th weeks (Gluck, 1971; Gluck et al, 1971; Nerola et al, 1974). The results vary according to the procedure.

Our findings are in accordance with Tiwari et al (1969) and Sharma et al (1981) who reported critical mean L/S values at 35-36 weeks gestation 2.30 and 2.207 respectively, where as in our study ratio was 2.175.

A linear correlation between level of surfactant and gestational age has been observed by various workers (Fozzard and White, 1972; Schulman et al, 1972; Bhagwani et al, 1972 a, b; Roux et al, 1973; Edward and Baillie, 1973;

Mukherjee et al, 1974; Nakamura and Roux, 1974; Sprouls et al, 1974; Rothbard and Dajus, 1974; Fairbrother et al, 1975; Hamilton et al, 1975; Reddy et al, 1978 and Bhushan et al, 1978).

Bubble Stability Test and L/S Ratio in Relation to RDS :

In our study, a definite relationship was observed between the results of bubble stability test and L/S ratio with the respiratory status of live-born neonates of 32-42 weeks gestation (Table XI). In 16 negative BST results, 14 had RDS (87.50%) and rest of 2 (12.50%) had transitional respiratory distress. Out of 53 positive BST result, no infant developed RDS, but intermediate results were not conclusive as out of 13 cases with intermediate BST result, 2 (15.38%) had RDS, 5 (38.46%) infants had transient respiratory distress, while 6 (46.16%) developed no respiratory distress.

Among 12 cases with less than 1.5 L/S ratio 100% were having RDS, while with 1.5 - 1.9 only 4 (26.67%) were without respiratory distress. Out of 55 cases of more than 2.0 L/S ratio, cent per cent were without RDS.

Clements et al (1972) observed the RDS in 100 per cent negative BST result and no RDS in 100 per cent positive BST result, intermediate BST results being not contributory. Almost similar results were observed by

Nobel et al (1972), Edward and Bailli (1973), Reddy et al (1978) and Khushan et al (1978). Our findings are also in almost accordance with them.

According to Whitfield et al (1972) L/S ratio above 2.0 can be regarded as safe from the point of view of pulmonary function. They had a ratio in the range of 1.5 - 2.0 as an index of transitional level of pulmonary maturity with chances of RDS after delivery. A ratio of 2.0 or above always indicate the neonate born at that time may be free from RDS (Gluck et al. 1974) as is evident from our study also (Table XI).

The overall conclusion which can be made from our study was that with positive BSF results and L/S ratio 2.0 or more, infants most certainly will not develop RDS. But negative and intermediate BSF results and L/S ratio less than 2.0 can give false interpretation in prediction of RDS (Table XI).

Fisher et al (1973); Bhagwansni et al (1973); Roux et al (1973); Edward and Bailli (1973); MacLennan and Roundurgh (1975) and Fairbrother et al (1975)have also stated that bubble stability test were reliable when positive but not completely reliable when negative and with intermediate BSF result, risk of immature level of surfactant was present.

Zachman et al (1973) found 3 cases of RDS with positive foam stability test. Donald and Calvin (1974) have found that with negative BST result chances of risk of RDS were nearly always present but with positive and intermediate BST results. RDS continued to occur specially in cases less than 34 weeks showing that besides surfactant deficiency intrapartum complications also play a significant role in causing RDS.

Bubble Stability Test and L/S Ratio in Relation to Birth Weight :

Relationship between bubble stability test, L/S ratio and birth weight was observed in 82 cases of normal pregnancy (Table XII). It was found that bubble stability test was of some value in predicting the birth weight of newborn. Negative BST results were mostly associated with birth weight less than 1.8 kg (90%) and positive results were associated with birth weight more than 2.2 kg (85.7%). Intermediate BST results had no definite relationship with birth weight. Our findings are almost similar to Bhushan et al (1978). They found that positive BST results predicted birth weight of 2.5 kg or more in 87.5% and negative results predicted birth weight less than 2 kg in 87.5% cases.

Infants with less than 1.8 kg birth weight had L/S ratio < 2.0 in cent per cent cases which showed

the reliability of negative BST result. L/S ratio 2.0 or more was found in 91.07% infants having birth weight more than 2.2 kg. Hence it was found that higher the birth weight more was the L/S ratio.

Spellacy and Buhi (1972) found significant correlation in L/S ratio and infant birth weight, while no constant relationship could be estimated between neonatal birth weight or gestational age and lung maturity by Tiwari et al (1979).

Bubble Stability Test and L/S Ratio in Various Abnormal Pregnancies :

In present series a total of 110 cases of various fetomaternal complicated pregnancy were studied. Respiratory status of live born was observed in 110 cases of abnormal pregnancy (31 to 43 and more weeks) and bubble stability test result and L/S ratio were determined (Table XV, XVI, XVII).

Hydramnios :- In our study, the results of bubble stability test in cases of hydramnios are highly misleading (Table XV, XVI, XVII), as referred by other workers also. Despite of 6 negative BST result and 4 intermediate result among 10 cases, no neonate developed RDS. Only one neonate developed transitional respiratory distress and one was still born. These negative BST results may be due to dilution factor. L/S ratio was

found to be more than 2.0 in 40% cases and others (60%) were having less than 2.0.

Nelson (1969) has found low level of surfactant (Total lipids, phospholipids and decreased level of lecithin in phospholipid fraction) in cases of hydramnios. Fisher and Sutherland (1973) observed similar results and explained that excessive volumes of amniotic fluid may have influenced phospholipids level and the interpretation of bubble stability test in the prediction of RDS. Rothbard et al (1974) also observed negative BST results in cases of polyhydramnios without development of RDS. Reddy et al (1978) reported false negative results in cases of hydramnios who had not developed RDS.

Toxaemia of Pregnancy :- A series of 20 cases of toxaemia of pregnancy were studied (Table XV, XVI, XVII). Comparatively early rise of mean L/S ratio in 33-34 weeks gestation were seen than corresponding group I values. Toxaemia of pregnancy may accelerate lung maturation, has been suggested by Aubry et al (1976); Bayer et al (1973); Gluck and Kulovich (1973); Gould et al (1977) and Morrison et al (1977). While Desburst et al (1973); Freeman et al (1974); Ekelund et al (1974); and Dayson et al (1975) have observed no acceleration of lung maturation in cases of pre-eclampsia.

In our study bubble stability test was found positive in 40.00% where only one neonate developed RDS, while negative BST result was in 25% with 2 neonate having RDS and intermediate BST result in 35% with one neonate having RDS. In this series of 20 cases where 4 neonates developed RDS, only in one case L/S ratio was more than 2.0. Our results are almost similar to Reddy et al (1978), they found one false negative result (in their series of 27 cases of high risk pregnancy with 5 neonates who developed RDS) in intrapartum eclampsia and explained on the ground that the half life of surfactant is shortened by pregnancy complications. Surfactant might have been present at the time of collection of fluid i.e. early in labour but would have been destroyed due to asphyxia as labour progressed.

Rh-immunization :- In pregnancies complicated by Rh-immunization 4 cases were studied. L/S ratio was more than 2.0 in 50% cases with positive BST result. No neonate developed RDS in the present study.

Many researchers have observed delayed lung maturation of fetus of Rh-immunised pregnancy (Whitfield et al, 1972; Gluck and Kulovich, 1972; Harding et al, 1973 and Aubry et al, 1976). In our study no difference from the control group could be found which may be due to the mild character of this disease.

secrete greater amounts of insulin, which then can inhibit surfactant production by preventing the corticosteroid induction of alveolar cells (Smith et al, 1975). Whereas in well controlled insulin dependent diabetes, the blood glucose of the mother is lower and more stable than in cases of chemical diabetes and fetal hyperglycemia does not occur so often.

Other cases :- Other complicated pregnancies including post maturity 14, Fetal distress 18, APH 16, Twins 14, Hydrocephalus 2 and Heart disease 6, were studied in present series. Out of 12 cases of negative BST result (Fetal distress 3, Twins 4, Hydrocephalus 1, Antepartum haemorrhage 4) 8 neonates were associated with RDS, 3 were still born and one developed transitional respiratory distress. In 55 positive BST result (postmaturity 14, Fetal distress 11, Twins 4, Heart disease 5, Diabetes 4, Rh-immunisation 2, APH 7 and toxaemia 8), no neonate developed RDS while variable results were obtained with intermediate group. When the test is positive, neonate will certainly not develop RDS, when intermediate neonate may or may not develop RDS, when negative neonate is definitely prone to develop RDS except in cases of hydramnios where false negative result may be obtained due to dilution of liquor. Roux et al (1973) also found that bubble stability test in high risk pregnancy reliably diagnosis pulmonary maturity only when it is positive.

negative and intermediate results are less reliable. Donald and Calvin (1974) suggested that in complicated pregnancy, negative SBT results were of predictive value but with positive and intermediate results, RDS could occur, because besides surfactant deficiency intrapartum events also modify the incidence of RDS.

In whole group of these miscellaneous complications, no significant difference in L/S ratio value were seen when compared with corresponding period of gestation in normal group except in hydrocephalus where delayed maturation occurred. It seems possible that the delayed maturation of this group is connected with metabolic changes in the overall fetal homeostasis.

Gluck and Kulovich (1973) found a linear correlation with gestational age only in normal pregnancy. They observed that abnormalities of pregnancy may affect lung maturation (production of surfactant). Some conditions like Hypertension, Toxaemia, Dysmaturity, Placenta problems like Retroplacental hemorrhage and Diabetes D and R (of white classification) accelerate lung maturation while Diabetes type A & B (of White classification) delay it. Risto Tuimala (1978) observed delayed maturation in group of complications including placenta praevia, Heart disease and Hydramnios.

RDS in Cases of Normal and Abnormal Pregnancy :

The higher risk of RDS was observed in normal pregnancy before 34 weeks gestation, but in abnormal pregnancy no significant difference was observed before and after 34 weeks of gestation (Table XIX). Risk of RDS was found to be more in complicated pregnancy after 34 weeks of gestation; overall incidence being 13.33% after 34 weeks, whereas 35.72% before 34 weeks.

The high risk of RDS before 34 weeks could be due to lack of production of surfactant which is responsible for lung maturation and also prevents the risk of developing RDS.

Cluck et al (1973) also found higher incidence of RDS in cases of abnormal pregnancy due to inhibition of surfactant production by hypoxia and acidosis. Aubry et al (1976) reported an incidence of 11.5% in abnormal pregnancy, which is in accordance to our findings. Whereas Robert et al (1976), Malan et al (1976) and Risto Tuimala (1978) reported the incidence of RDS in 2.6%, 1% and 6.1% respectively in cases of abnormal pregnancy.

RDS in Relation to Route of Delivery :

Significant difference was observed in the risk of RDS according to route of delivery (Table XX). Incidence was found to be 16% in vaginally delivered cases, whereas in caesarean section it was 26.19%.

Similar results were found by Usher et al (1971); Gabert et al (1973); Crum et al (1976) and Lofstrand (1976). They observed that incidence of RDS was exaggerated by caesarean section delivery. Risto Tuimala (1978) have shown significantly higher incidence of RDS in the caesarean section group (15.8%) than in vaginally delivered group (3.8%) as in our study. While Donald and Freeman (1973) and Reddy et al (1978) have observed no detectable difference in the incidence of RDS and route of delivery.

Apgar Score and RDS :

Risk of RDS is significantly related to Apgar Score (Table XXI). Low Apgar score was nearly always found to be associated with higher incidence of RDS. Among infants developing RDS, 41.46% had Apgar score 7 or below and 2.5% had Apgar score 8 or more. Avery and Mead (1959); Donald and Freeman (1973); Avery (1973); Donald and Calvin (1974) and Avery (1975) have also suggested that low Apgar directly influenced the incidence of RDS as observed in our study.

Low Birth Weight Infants and RDS :

Risk of RDS was found to be inversely related to birth weight of neonates. With birth weight 2.6 kg and more, chances of developing RDS was 4.34% as compared

to the incidence of 27.96% in cases with birth weight below 2.6 kg (Table XXII). Chances of RDS were nil in infants weighing more than 3 kg at birth.

Donald and Freeman (1973) have also observed more chances of RDS in low birth weight infants, but a birth weight of more than 2.5 kg does not necessarily excludes RDS according to them. Gluck and Kulovich (1973) have suggested that maturation of lung is independent of birth weight and found low incidence of RDS in low birth weight babies than true prematures. Risto Tuimala (1976) observed higher percentage of RDS in small for dates babies (16.7%) as compared to normal weight (5.6%).

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CONCLUSIONS

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In the present study, bubble stability test and lecithin/sphingomyelin ratio in amniotic fluid were determined in normal and abnormal pregnancies for antenatal prediction of fetal lung maturity. The results of these tests were analysed and their correlation to duration of pregnancy, respiratory status of new born, maternal complications, birth weight and Apgar score of neonate were studied.

In brief it is concluded that -

1. A good correlation between gestational age and results of bubble stability test is present. After 36 gestational weeks, most of the cases show positive test and before 34 weeks no case is found to have positive BST result.

2. Lecithin level in amniotic fluid shows gradual rise throughout pregnancy upto 35 weeks, when there is a surge at 36 weeks of gestation followed by again gradual rise till term.

3. Sphingomyelin shows gradually decreasing value throughout pregnancy.

4. Lecithin is the principal phospholipid of late pregnancy while sphingomyelin appeared to be the principal phospholipid of early pregnancy.

5. L/S ratio shows a sustained rise throughout pregnancy, with a sudden and marked rise between 35-36 weeks, thereafter the rise is again gradual.

6. The positivity of bubble stability test and sudden rise of L/S ratio between 35-36 weeks of gestation signifies the fetal lung maturity.

7. Bubble stability test and L/S ratio show a good correlation (95.23%) as till 30th week cent per cent cases are of negative BST result and L/S ratio less than 2.0, while at 37-38 weeks of gestation, 80% showed positive BST result and L/S ratio more than 2.0 in 84%, a level safely indicating pulmonary maturity.

8. A correlation between bubble stability test, L/S ratio and respiratory status of live born has been discussed and it is suggested that negative and positive BST result and L/S ratio more than 2.0 can be used as an index of fetal pulmonary maturity. Intermediate BST results are not conclusive.

9. On studying the bubble stability test and L/S ratio in relation to birth weight of live born in normal

cases, positive BST result and L/S ratio 2.0 or more are found to be associated with birth weight of 2.5 kg or more in most of the cases.

10. Our data denotes the predictive value of bubble stability test and L/S ratio in relation to Apgar score of the neonate. Apgar score of 8 or more is associated with positive BST result and L/S ratio 2.0 or more as safe from the point of view of pulmonary function.

11. In toxaemia of pregnancy, values of L/S ratio are higher as compared to normal pregnancy group in corresponding gestation period. This denotes significant pulmonary maturity acceleration in toxaemia.

12. In hydramnios false negative BST results are observed in 40%. No infant developed RDS inspite of no positive BST result. The negative BST result may be due to dilution factor of amniotic fluid.

13. In Rh-immunization, L/S ratio and bubble stability test show no significant difference from control group, which may be due to mild character of this disease.

14. Higher values of L/S ratio with positive BST results are obtained in diabetes and this indicates accelerated lung maturation.

15. Phospholipid level in amniotic fluid is directly proportional to lung maturity of fetus as more than 3.0 L/S ratio with positive BMT result were observed in all cases and no infant developed RDS.

16. In other complications like heart disease with pregnancy, twins, fetal distress, antepartum haemorrhage and hydrocephalus, no significant difference from normal group was observed.

The present study shows that bubble stability can be used as screening procedure as it is cheaper and easier to perform and more elaborate L/S can be estimated when very small bubbles are obtained or clumping occurs, to differentiate the possibly immature fetus from likely mature fetus and in those cases showing a deviation from normal.

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ABBREVIATIONS

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AF	Amniotic Fluid.
BST	Bubble Stability Test.
DPL	Dipalmitoyl Lecithin.
HMD	Hyaline Membrane Disease.
L/S	Lecithin / Sphingomyelin.
LSAR	Lecithin-Sphingomyelin Spot Area Ratio.
PG	Phosphatidylglycerol.
RDS	Respiratory Distress Syndrome.
TND	Transient Respiratory Distress.
TLC	Thin Layer Chromatography.

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